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AMINO ACID HANDBOOK

Methods and Results of Protein Analysis

PROFESSOR AND MRS. LAFAYETTE B. MENDEL AT HOME, DELHI, NEW YORK.

AMINO ACID HANDBOOK

METHODS AND RESULTS
OF PROTEIN ANALYSIS

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BANNERSTONE HOUSE
301-327 East Lawrence Avenue, Springfield, Illinois, U.S.A.

Published simultaneously in the British Commonwealth of Nations by
BLACKWELL SCIENTIFIC PUBLICATIONS, LTD., OXFORD, ENGLAND

Published simultaneously in Canada by
THE RYERSON PRESS, TORONTO

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Library of Congress Catalog Card Number: 56-9104

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F8,3;R:f8

N56

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Printed in the United States of America

Objectives

WE HOPE to do two things in this monograph. First, to describe in detail tried and proven examples of the three most widely used methods of amino acid analysis, *i.e.*, by microorganisms, by column chromatography, and by paper chromatography. It is hoped that sufficiently detailed directions will be given for each of these methods so that the analyst will be able to determine all of the amino acids which commonly occur in proteins without the need of recourse to the original literature. In contrast to our previous monographs on amino acids (Block and Bolling, 1945, 1951) no attempt is made to describe the many modifications proposed by various investigators although some comments are made concerning difficulties and possible pitfalls which may be encountered during the analysis.

The second part of the monograph is devoted to a tabulation of the amino acid composition of proteins, biologically active polypeptides and foods. The majority of the values for the amino acids in proteins and foods have been calculated as grams of amino acid per 16.0 grams of nitrogen in order to facilitate comparison between the results of different analysts on various preparations and to permit more ready use of the data for nutritional calculations.

ABBREVIATIONS

Mtd.	Method
N	Nitrogen
S	Sulfur
M.R.	Molar Ratio
ARG	Arginine
HIS	Histidine
LYS	Lysine
TYR	Tyrosine
TRY	Tryptophan
PHE	Phenylalanine
CYS	Cystine
MET	Methionine
SER	Serine
THR	Threonine
LEU	Leucine
ISO	Isoleucine
VAL	Valine
GLU	Glutamic acid
ASP	Aspartic acid
GLY	Glycine
ALA	Alanine
PRO	Proline
HYD	Hydroxyproline

Preface

"The current trend of the investigation of the chemistry of nutrition is emphasizing the significance of the amino acids as the fundamental factors in all problems in which hitherto the role of proteins has been involved. . . . Obviously the relative values of the different proteins in nutrition are based upon their content of these special amino acids which cannot be synthesized in the animal body and which are indispensable. . . ." These views, set forth by T. B. Osborne and L. B. Mendel in 1914, cover the accepted facts on protein nutrition today. The extension of the pioneer experiments of Willcock and Hopkins, Osborne and Mendel, and others were brought to a successful conclusion by the well-known results of one of Professor Mendel's distinguished students, Professor W. C. Rose of Illinois.

With the proof of the essential nature for animal nutrition of methionine, histidine, lysine, tryptophan, phenylalanine, threonine, leucine, isoleucine, and valine, and the special importance of cystine, arginine, tyrosine, and glycine as shown by W. C. Rose, H. J. Almquist, R. W. Jackson, H. H. Mitchell, and others, the nutritive evaluation of protein foods, based on their comparative amino acid composition became a possibility. A reasonably accurate knowledge of the amino acid composition of a protein permits an approximation of its nutritive value and, more important, allows the choosing of different proteins so that they become mutually supplementary. The formulation of diets based on amino acid composition results in a great saving of time and cost over the long and tedious trial-and-error type of animal test employed heretofore.

If the amino acid requirements of an animal are known, even approximately, the proper quantities and combinations of food proteins can be chosen, provided that their essential amino acid composition has been estimated by methods of comparable accuracy. Evidence is beginning to accumulate concerning the special importance of certain amino acids in pre- and post-operative treatment, in wound healing, in blood regeneration, in learning processes, and even in the etiology of mental disease. Again, a knowledge of the amino acids present in available proteins may permit the use of relatively inexpensive sources rather than the more costly purified amino acids. Amino acid analysis has revealed rich sources of specific amino acids in protein products which were heretofore unsuspected. Protein analysis is also valuable in revealing new uses of the protein-containing by-products of industry, and, in quickly revealing specific deficiencies in natural and prepared foodstuffs.

The degree of experimental accuracy in the amino acid analysis of the naturally occurring heterogeneous proteins, which may vary within wide limits because of preparative difficulties as well as changes in the amino acid composition of the tissue or organism itself, does not need to be as high as that required in a study of the molecular structure of those rare proteins which have been shown to be homogeneous substances (*cf.*, Colvin, 1954). Although in all analytical work, one must strive for the determination of absolute values, nevertheless, much valuable information of a permanent nature can be achieved by comparative amino acid analyses, especially in relation to protein nutrition.

This monograph gives many of the analytical values from the literature as well as some hitherto unpublished results of our own. Those proteins for which only a few analytical values are available have been omitted.

Many of the thoughts and ideas expressed in this monograph are the result of the kind suggestions and criticisms of various scientists, including Drs. Max Bergmann,* R. Keith Cannan, George R. Cowgill, Israel S. Kleiner, Icie Macy-Hoobler, William A. Perlzweig,* Carl L. A. Schmidt,* M. X. Sullivan, Donald D. Van Slyke, Hubert B. Vickery, and above all, Professor Lafayette B. Mendel.*

Although a survey of the literature on protein analyses has been attempted, the authors realize that many valuable contributions, especially those in foreign journals and in periodicals not devoted primarily to physiological chemistry, have been omitted unintentionally. It is also probable that, due to the many calculations and transpositions of data from the literature, which were necessary in the preparation of this monograph, numerical errors have been made. The authors welcome the correction of any erroneous statements or calculations and hope that readers will be so kind as to call their attention to important papers on this subject which have been omitted.

We wish to acknowledge our indebtedness to the writers and publishers of the various scientific books and journals from which the greatest part of the descriptions of experimental details, the analytical results, and almost all the figures and diagrams were taken. Although specific credit is given in the text or bibliography, it is hoped that any omissions will be brought to the authors' attention for rectification.

---

*Deceased.

R. J. B.

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PART B

THE AMINO ACID COMPOSITION OF PROTEINS

THE AMINO ACID COMPOSITION OF PROTEINS (For table of Contents see page 249).

AMINO ACID HANDBOOK

Methods and Results of Protein Analysis

CHAPTER I

Introduction

THE NUTRITIVE importance of proteins and the dependence of animals on plants for these substances were first pointed out by G. J. Mulder around 1840 in *The Chemistry of Animal and Vegetable Physiology.* He said, "In both plants and animals a substance is contained, which is produced within the former, and is imparted through their food to the latter. . . . It is unquestionably the most important of all known substances in the organic kingdom. Without it no life appears possible on this planet. Through its means the chief phenomena of life are produced."

A few years later Boussingault, writing in the *Économie Rurale* (Paris, 1851) said, "The alimentary virtues of plants reside above all in the nitrogenous substances, and consequently their nutritive potency is proportional to the quantity of nitrogen entering into their composition."

The views of Boussingault were strengthened by Liebig, so that in spite of much evidence against the nutritive equivalence of plant and animal proteins, the view that protein nutrition was essentially nitrogen nutrition remained dominant until 1897 when Rubner recognized that proteins of varying origins were *not* of the same value in nutrition and that therefore there was no one protein minimum but as many minima as there were proteins.

This rather unpromising outlook for research on the biological evaluation of proteins was changed to an hypothesis which could be placed on a rational experimental basis a few years later by Osborne (1907), who concluded his monograph on *The Proteins of the Wheat Kernel* with the following:

> "The proportion of lysine [in wheat gluten] is likewise small, especially compared with that obtained from the leguminous seeds. The amount of histidine, however, does not differ very greatly from that of other seed proteins. What significance these differences have in respect to the nutritive value of these different proteins must be determined by future investigation, for it has only very recently been discovered that such differences exist.
>
> "That a molecule of gliadin can have the same nutritive value as one of casein would seem impossible if one molecule of food protein is transformed into one of tissue protein, for in the former lysine is wholly

> lacking. . . . It would seem probable that either the animal requires a variety, so that the relative proportion in which the amino-acids are available for its use shall correspond more nearly to its requirements, or that only a small part of these amino-acids are converted into its tissue proteins and the rest oxidized as such. It is possible that feeding experiments with proteins of known character in respect to the relative proportions of their decomposition products will throw light on these important questions."

About the same time, Willcock and Hopkins (1906) observed that when casein was the sole source of protein for young mice good growth was achieved. However, if the mice were fed a diet containing zein or zein plus tyrosine, they almost immediately lost weight and shortly died. If, however, tryptophan were added to the zein diet, the animals lived for as long as thirty-two days.

At this time, the general impression arose that proteins of plant origin were inferior to those of animal origin for purposes of nutrition. This idea is still prevalent, for at a conference sponsored by the Children's Fund of Michigan in 1944, the following question was asked: "Do you consider vegetable and animal proteins interchangeably adequate in diets for human beings"? The correct answer was given by the speaker as follows: "My view is that an essential amino acid is essential, whether it comes from animal or vegetable protein." This question was the result of the widespread impression of a fundamental difference between proteins of plant and animal origin even though Osborne (1908) said: "On chemical grounds there is no more reason for dividing the proteins into two groups of animal and vegetable protein than there is for making a similar distinction between carbohydrates."

Shortly thereafter Osborne and Mendel inaugurated their classical studies on the nutritive value of purified proteins and protein-containing foods. These and other investigations formed the basis for the current ideas on the nutritive values of protein foods, which were summarized by Mendel (1923) as follows:

> "The proportion of protein in the diet may determine whether larger or smaller absolute amounts of the nitrogenous foodstuffs are consumed; but the actual intake of these is also modified by the character of the non-protein ingredients. The individual instinctively strives to satisfy its calorific needs. A diet rich in fats is consumed in smaller quantity than one poor in fats, consequently, the absolute protein intake may vary independently of its concentration or percentage content in the food. When the absolute intake is small the 'law of minimum' may come into play to limit the efficiency of the whole because

> of the relative shortage of some essential amino-acid. Thus the protein casein yields relatively little cystine. So long as casein appears in the diet in abundance no deficiency appears; but when the consumption of the proteins is reduced a shortage of the least abundant essential may manifest itself. . . .
>
> "Conversely, when an animal ingests a very large quantity of some protein poor in an essential unit, the absolute amount of the latter thereby available from the great abundance of its precursor may suffice to promote nutritive effects that fail to appear on a lower plane of protein intake."

McCollum, as quoted by Mendel (1929, p. 121), too, remarked that the investigations carried out during the period between 1910 and 1920 on protein foods of plant origin "leave no room for doubt that all the amino-acids necessary for the nutrition of an animal are contained in the proteins found in each of these foods. Certain of these are, however, present in such limited amounts as to restrict the extent to which the remaining ones, which are more abundant, can be utilized. It is for this reason that these proteins are of relatively low biological value unless supplemented by proteins from other sources, the constitution of which is such as to make good their deficiencies. The proteins of the cereal grains are of lower value than those of milk and eggs, or than those of certain mixtures obtained by combining two or more of these seeds."

Until the beginning of the twentieth century, it was generally believed that only intact proteins could be satisfactorily utilized for purposes of nutrition. In 1902, however, Loewi was able to maintain dogs in nitrogen equilibrium on pancreatic autolysates. The following year, Henderson and Dean (1903) attained nitrogen equilibrium in a dog by feeding a sulfuric acid hydrolyzate of meat. Henriques and Hansen (1905) prepared polypeptide-free amino-acid solutions by first digesting the protein with trypsin and erepsin and then heating the digest with 20% sulfuric acid. It was found that if the first product gave a pronounced tryptophan reaction it would support nitrogen equilibrium. If, however, a single amino acid, tryptophan, were lacking, nitrogen equilibrium could not be attained. These experiments formed the basis for the now currently accepted idea that protein nutrition is essentially amino acid nutrition.

From these investigations, and many others, the concept was established that the amino acids can be divided, roughly, into two groups which are called essential (indispensable) and nonessential (dispensable). Essential amino acids for growth are those which cannot be synthesized by the animal at a rate sufficient for optimum growth out of the materials ordinarily available in the diet. The term "indispensable" amino acid has a significance only when qualified as to species, nutritional state and age of the animal, a fact which is often overlooked (Rose, 1938).

The elucidation of the specific nutritive roles played by certain amino acids for growth, reproduction, lactation and maintenance indicated the need for information concerning the amino acid composition of food proteins as they are commonly consumed by man and animals, rather than knowledge of the amino acid composition of certain purified proteins, which may account for only a small percentage of the total proteins of the food.

ANALYTICAL DIFFICULTIES

There is one stumbling block in the accurate determination of amino acids in proteins which must be constantly emphasized. Amino acid methods, with few exceptions, require hydrolysis of the protein before they can be applied. The susceptibility of each and every amino acid to loss during hydrolysis is different and differs not only with the conditions of hydrolysis, but more so with the presence or absence of other substances in the hydrolysis mixture. Thus, various investigators have shown that cystine and especially cysteine may be destroyed during acid hydrolysis when carbohydrates are present, but not in their absence.

Attempts have been made to evaluate hydrolytic losses by adding the amino acid under investigation both before and after hydrolysis. The apparent destruction during hydrolysis can then be determined by the difference in the analytical results obtained. This requires the tacit assumption that the amino acid added to the protein reacts during the hydrolysis in exactly the same way as its analogue in the peptide linkage, a fact which is known to be untrue in the case of certain amino acids. However, this procedure is, it is believed, the best approximation that can be made at the present time whenever hydrolysis is required.

It is obvious that the goal of protein analysts is the development of methods which do not require preliminary hydrolysis. Certain beginnings along this line have been made. Thus, Holiday (1936) and other investigators have been able to estimate the quantities of tyrosine and tryptophan in the intact protein by spectrographic methods. Bates (1937) used a modification of the Voisenet-Rhode *p*-dimethylaminobenzaldehyde method for estimating tryptophan in proteins. Mirsky and Anson (1934-35) and others, have utilized the reducing action of the sulfhydryl group of cysteine to estimate the quantities of this amino acid and of cystine in proteins without hydrolysis.

AMINO ACID ANALYSES

1. Chemical Methods

For the first 40 years of the present century, knowledge of the amino acid composition of proteins depended almost entirely on purely chemical procedures. These consisted either in isolating and weighing the individual amino acids or causing them to react specifically with a compound to form a colored product. The intensity of the color of this product was then estimated by comparative colorimetry. In a few instances, the amino acids were determined by oxidation to more readily measurable compounds. (See Block and Bolling, 1951.)

2. Physico-chemical Methods

A compound which has an abnormal isotope content is inseparable from its normal analogue by the usual laboratory procedures. In the case of a compound containing N^{15} the proportion of this abnormal isotope can be determined by means of the mass spectrograph. The principle of the method of estimating a given amino acid in a protein depends on adding to an hydrolyzate of the protein a definite amount of a pure preparation of the amino acid containing a known percentage of N^{15} and then to determine the proportion of abnormal to normal N in a pure specimen of the amino acid isolated from the hydrolyzate.

This procedure, which appears to be the most accurate method now available for the determination of amino acids in protein hydrolyzates, is limited only by the availability of the equipment, the isotope-containing amino acids and technical ability. It suffers from the same disadvantages of more or less extensive losses of amino acids during hydrolysis that plague the more widely used procedures.

3. Biological and Microbiological Methods

Although biological methods for amino acid analysis were introduced by Osborne and Mendel in 1914 and are still in use for the estimation of amino acids in proteins and foods, the results so obtained are seldom considered to be sufficiently reliable, because of the limited number of test subjects employed, to permit a numerical expression of the amino acid composition of the protein. It should be recalled, however, that the large discrepancy between the "cystine" value of casein as determined by the rat growth method (Sherman, 1925) and that found by chemical procedures, suggested to Jackson (1931; 1932) that methionine may be able to assume all or part of the nutritive functions of cystine. This led to the discovery of the essential nature of methionine.

The determination of an amino acid based upon its decomposition by a specific enzyme system also is not new. Thus, Jansen (1916) used the formation of ammonia by the successive action of arginase and urease as a specific method for the determination of arginine.

Since 1943, however, there have been great developments in the use of biological methods employing microorganisms and specific enzyme systems for the routine estimation of all the known amino acids. These methods, furthermore, are fundamentally so similar in technique that once a procedure is learned for any one amino acid, it can be applied with equal facility to any other for which suitable conditions have been found. The value of this, especially for routine analysis, is apparent.

However, it should be remembered that the growth of living organisms is subject to many more extraneous influences than are chemical reactions or physical measurements. The presence or absence of vitamins, of lipoid substances and even of compounds closely related to the amino acid under examination, may greatly influence the growth of the test organism, and may even determine the "essentiality" of an amino acid for that organism. It should be recalled that, with few exceptions, microbiological methods require complete hydrolysis of the protein for uniform results and are, therefore, subject to the same hydrolytic losses which have been discussed before.

4. Chromatographic Methods

These procedures have largely replaced the older chemical methods since 1950. They are of special value where highly accurate determinations of all the amino acids present in an isolated protein are to be made (column chromatography) or when one wishes to compare several or all of the amino acids in a number of proteins or foods (paper chromatography).

AMINO ACID COMPOSITION OF FOOD PROTEINS

The inherent error in calculating protein from nitrogen, especially in certain plants, such as yeasts and soybeans, has long been known.

If the plant proteins are isolated in the purified form and then analyzed for their constituent amino acids, and if the results so obtained are used to calculate the amino acid composition of the tissue from the nitrogen content of the latter, the error may be considerable. But if the tissue is itself analyzed for the amino acids, this error is minimized. Results so obtained show the approximate amino acid composition of the proteinaceous substances irrespective of the presence of nonprotein nitrogen. The majority of the analyses summarized here are intended to be used to indicate the amino acid composition of foods and not for consideration of protein structure.

Inspection of the data in this monograph indicates that the heterogeneous proteins of actively metabolizing living matter, whether from plants or animals, have approximately the same overall pattern of amino acids.* On the other hand, special proteins, such as the keratins, hemoglobins, serum proteins, and hormones, and the storage proteins of seeds, are not as well-balanced with respect to their amino acid distribution.

METHOD OF PRESENTATION OF ANALYTICAL DATA

In the interest of uniformity and in order to facilitate comparison of data on the same protein by different investigators, the analytical figures given in this monograph have been calculated to 16.0 grams of nitrogen or as the molar ratios, wherever possible. In certain cases where nitrogen values were not given but the determinations were calculated on the basis of the moisture, fat, and ash-free preparation, the data have been recalculated to 16.0 grams of nitrogen using a generally accepted value for nitrogen which is always indicated by placing the nitrogen value in parentheses. In some cases, the value (16.0) gm. of nitrogen is used to indicate that the amino acid values are given exactly as reported. This notation does not, therefore, always mean that the protein in question was deemed to have 16.0% nitrogen. It is recognized that this procedure may introduce a certain error, but it is believed more suitable than other methods of presentation.

As all the data in the tables are calculated on the basis of 16 grams of nitrogen, it is only necessary to know the nitrogen content of the protein in order to recalculate the data in the tables to give the approximate amino acid composition of the preparation. If the

---

*Cf. *A Statistical Evaluation of the Kinship of Protein Molecules,* Fox (1955).

protein contains 18.6% of nitrogen, then the values in the proper table are multiplied by the factor

$$\frac{18.6}{16.0} \text{ or } 1.16$$

If the nitrogen of the product is only 12.2%, then the amino acid figures are multiplied by the factor

$$\frac{12.2}{16.0} \text{ or } 0.76$$

CHAPTER II

Preparation of the Sample for Analysis

GENERAL PROCEDURE

As a preliminary step in the amino acid analysis of any material it is recommended that its protein content (nitrogen × 6.25) be determined. If the per cent nitrogen is 13.5 or above, the material can be considered relatively pure protein and can be hydrolyzed without further treatment. However, if the nitrogen content falls below the indicated limit, as it usually would in foods, feeds and similar materials, it is essential to remove nonprotein substances following the methods outlined in this Chapter. The success of the analysis and the accuracy of the results depend in part on the removal of interfering substances such as lipids and carbohydrates. Thus, in brief, the usual procedure follows this pattern:

1) Determination of nitrogen content of sample; if high protein content is indicated, material is hydrolyzed without further preparation.

2) Removal of lipids by solvent extraction.

3) Removal of carbohydrates either by enzymatic digestion or chemical extraction.

4) Determination of nitrogen to establish final purity of protein.

5) Desalting of sample, if high salt content is indicated.*

6) Hydrolysis of protein to its amino acid components.

7) Determination of nitrogen of hydrolyzate and dilution to desired volume.

8) Determination of amino acids by the method of choice: chemical analysis, microbiological assay, paper chromatography or column chromatography.

DETERMINATION OF NITROGEN

1. Semi-Micro Kjeldahl Method

Reagents

Digestion mixture. 100 g. of K_2SO_4 + 10 g. $CuSO_4$. Grind in a mortar so that lumps of $CuSO_4$ are powdered. Mix well.

---

*In practice desalting is only applied before hydrolysis when one wishes to analyze for the amino acids in dialyzable peptides and free amino acid solutions.

Selenium granules. Se-coated chips (Hengar Co., Phila.)

Concentrated sulfuric acid. H_2SO_4, A.R.

Hydrogen peroxide. 30% H_2O_2, A.R., nitrogen-free.

Boric acid solution. 2% H_3BO_3 in distilled water.

Indicator[*] for titration. 200 mg. methyl red + 100 mg. methylene blue. Make up to 200 ml. with absolute ethyl alcohol. (Keep cold.)

Sodium hydroxide. NaOH pellets, A.R.

Standard acid solution. 0.02 *N* HCl or $KHIO_3$ prepared as follows: make an approximately 0.02 *N* solution of the acid of choice. Weigh samples of dried glycine and ammonium sulfate to contain 1-3 mg. N. Digest and distill according to the procedure given below. Titrate with acid solution to be standardized.

$$\text{Factor} = \frac{\text{mg. N}}{\text{ml. acid}}$$

Use the average value of the factors obtained from several determinations for calculation of nitrogen in unknown.

Apparatus:

100-ml. Kjeldahl flasks.

Pyrex air-cooled condensers for distillation, bent in U-shape (Folin, 1919).

250-ml. Pyrex Erlenmeyers for receiving flasks.

Procedure: Weigh the sample, transfer quantitatively to Kjeldahl flask, or pipette aliquot of liquid into flask. The sample should contain 1-3 mg. of nitrogen. Add approximately ¼ teaspoon of digestion mixture, 1 Se-coated granule, and 2.5 ml. of concentrated H_2SO_4. Place on digestion rack and heat for 15-20 minutes. Add 0.5 ml. of H_2O_2 carefully. Return to heat and add 0.5-1.0 ml. more of H_2O_2 in 0.5 ml. portions as it is needed. Turn flasks occasionally to aid complete digestion of all particles. When mixture is clear green, heat for 30-60 minutes longer or if necessary, heat 7 hours longer.* Samples containing much carbohydrate tend to foam considerably and must be watched until foaming subsides. It may be necessary to wash down the neck of the flask with distilled water if excessive foaming has caused the sample to move up the neck of the flask. When digestion is complete, allow flasks to cool and add 35-40 ml. of distilled water, mixing until the salts are all in solution. Cork flasks and cool in refrigerator or place flasks in ice water until they are cold. Meanwhile prepare receiving flasks for distillation; into each place 30 ml. of the boric acid solution and 3-4 drops of the indicator. Rinse the inside of the condensers with distilled water and place the receiving flasks under the end of the condenser so that the condenser end is just under the surface of the boric acid solution but does not touch the bottom of the flask. When the contents of the digestion flasks are well-chilled, add approximately ½ teaspoon of NaOH pellets. Add them quickly and do not shake the mixture. Attach the flask to the rubber connection on the condenser as quickly as possible and then shake the mixture. Heat gently. Distill for 15 minutes, then lower the receiving flasks so that the end of the condenser is out of the solution and continue distilling for 5 minutes longer. The condenser above the receiving flask should be hot near the top but not at the bottom of the arm. If the distillate is hot when it reaches the boric acid, the acid thus becomes warm and loses its ability to retain the ammonia. Titrate the ammonia with the standard acid and calculate as follows:

---

[*] Sher (1955) suggests the following indicator: dissolve 350 mg. bromocresol green in 10 ml. of 95% ethyl alcohol, add 1.0 ml. of 0.5 *N* NaOH and 200 ml. of water. Then add 22.1 ml. of 1% aqueous New Coccine (Ansco, Binghamton, N. Y.) and 750 mg. of *p*-nitrophenol in 5 ml. of ethyl alcohol. Dilute to 250 ml. Indicator should be light grey in pH 4.6 buffer. Dilute 15 ml. of indicator to 2 l. with 2% boric acid.

$$\text{ml. acid} \times \text{Factor} = \text{mg. nitrogen in sample}$$

2. Micro Kjeldahl Method for 0.5-15 γ of Nitrogen

This procedure (Boissonnas, 1953) is essentially the same as described above except that the ammonia is measured colorimetrically with ninhydrin (see Column Chromatography, Chapter VI) and smaller quantities of sample and reagents are used (*cf.*, Lanni, 1950 who uses the Nessler method to determine the ammonia).

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*Chibnall (1943), in confirmation of the earlier investigators (Osborne, Sørensen, etc.), has again proved that proteins and protein hydrolyzates, especially those rich in lysine and histidine, must be digested eight hours or more after the Kjeldahl liquid is clear even when catalysts and micro quantities are used.

3. Rapid Kjeldahl Method

This procedure was developed by Perrin (1953).

Reagents:

Silica granules. Not selenized.
Mercuric oxide. HgO, A.R.
Boric acid. 4% H_3BO_3
Zinc. 20 mesh Zn
Indicator. 5 parts of 0.2% bromocresol green + 1 part of 0.02% methyl red in ethanol.

Method: Mix in Kjeldahl flask the following: 500-1000 mg. of sample, 6 silica granules, 1.3-1.5 g. of HgO, 12 ± 0.5 g. of K_2SO_4 and 15 ml. conc. H_2SO_4. Digest at a low heat for 5 minutes or until frothing stops. Then increase heat to "full" for 8-12 minutes. Cool, add 200 ml. of water, 25 g. of NaOH, 5 g. of $Na_2S_2O_3$, and 500 mg. of Zn. Distill. Determine ammonia in any convenient fashion.

REMOVAL OF LIPIDS

The lipid-containing material is extracted with approximately 10 times its weight of the following solvents in succession; acetone (twice), hot benzene:absolute ethanol (95:5 v/v) (twice), hot ethanol and ether. Each solvent is removed from the proteinaceous residue by filtration (or centrifugation) and drying in air.

REMOVAL OF NUCLEIC ACIDS

Tissue nucleic acids may be removed from lipid-free tissues by digestion with 10% sodium chloride at 85° C. for six hours (Weiss, 1955). The sodium chloride is removed by washing with water and the residue dried with hot acetone (*cf.*, Decker, 1944).

REMOVAL OF CARBOHYDRATES

The presence of carbohydrates results in from negligible to very large losses of amino acids during hydrolysis, especially with mineral acids. It is therefore necessary to separate as much of the contaminating carbohydrates as possible from the protein. Unfortunately, there is no procedure that can be applied under all conditions which will result in the desired goal; however, some of the most useful methods will be described.

1. Digestion of the Starch With Amylolytic Enzymes

A large proportion of the starch in cereal grains may be removed as follows. Ten grams of grain are ground to a fine flour, if necessary, and triturated with 90 ml. of hot water until no lumps remain. The pH is adjusted to 4.5 with a little 5 *N* acetic acid and the suspension is heated in boiling water for one hour. Then 180 ml. of cold water are added to the suspension and the pH is adjusted to 7.0 with 5 *N* NaOH. After cooling to 37° C., 10-25 ml. of fresh human saliva, clarified by centrifugation, are added. The starch is digested at 37-40° C. over-night in the presence of toluene as the preservative. At the end of the digestion period, the insoluble protein residue is removed by centrifugation and thoroughly washed with hot water, acetone, hot benezene:ethanol = 95:5, ethanol and ether. The increase in nitrogen achieved by this procedure is 3 to 5 fold (Block and Bolling, 1951).

2. Extraction of Starch With Hydrochloric Acid

Defatted flour is extracted with cold 21% HCl. As the HCl extract contains some soluble protein, the starch is precipitated with an equal volume of ethanol and the filtrate is added back to the proteinaceous residue (Csonka, 1939).

3. Extraction of Starch With Trichloroacetic Acid

Wheat flour is boiled with 100 volumes of 5% trichloroacetic acid to remove the starch and to precipitate the protein. This procedure increases the nitrogen of the residue approximately 3 fold (Wöstmann, 1950).

4. Extraction of Protein With Dilute Hydrochloric Acid

Ground dry grasses are boiled with 4% hydrochloric acid for 48 hours under reflux. This gentle hydrolysis separates the proteins from the more resistant fiber (Miller, E. J., 1935).

5. Extraction of Protein With Formic Acid

Extraction of plant materials high in fiber with 90% formic acid either at room temperature or on the steam bath has been used to solubilize the proteins. The protein solution, which usually contains some carbohydrates soluble in formic acid, is further purified by precipitating the carbohydrates with 2 volumes of ethanol (Wilkins, 1937; Mazur, 1938; Albanese, 1944).

REMOVAL OF PROTEIN FROM AMINO ACID SOLUTIONS

Where an investigation of the free amino acids and peptides in plant or animal tissues is to be made, as many of the other constituents as possible must be removed. Dialysis or precipitation with trichloroacetic acid, tungstic acid, ferric hydroxide, zinc sulfate: barium hydroxide at pH 7.2-7.6, perchloric acid, acetone, and ethanol have been used (*cf.*, Agren, 1949; Block, 1934; de Verdier, 1948; McFarren, 1951; Roberts, 1950a and many others).

One method is to grind the tissues with sufficient absolute ethanol in a Waring Blendor so that the final concentration of alcohol is 80% by volume. The insoluble material is removed by filtration and washed with 80% ethanol. Then, 3 volumes of chloroform are added to each volume of the ethanol extract. After thorough shaking the resulting aqueous layer (upper) is removed and concentrated to the desired volume (Awapara, 1948).

Another procedure is to precipitate the protein with a slight excess of $HClO_4$ (1 or 2 *M*). The excess $HClO_4$ is then removed by the addition of the minimum quantity of potassium acetate (Neuberg, 1944) which results in the formation of the insoluble $KClO_4$.

If trichloroacetic acid is used as the protein precipitant, the excess acid may be neutralized to pH 6 with *N*-trioctylamine or the like, and the salt extracted with chloroform and ether (Hughes, 1951; Smith, E. L., 1948).

DESALTING OF AMINO ACID SOLUTIONS

A large excess of inorganic ions will interfere with the preparation of "good" chromatograms, especially with those amino acids of low R_f values in phenol. Several procedures have been proposed for their removal, but no one of these methods is entirely satisfactory. Some of the available desalting procedures will be described.

1. Ion Exchange Methods

All the cations in the solution, which include all amino acids except the strongly acidic taurine, are adsorbed on a cation exchange resin (Duolite C-3, H^+ cycle; Amberlite IR-120, H^+ cycle, etc.). The column is washed with water until all anions and nonionic material (carbohydrates, etc.) have been removed. The amino acids and the weaker cations are then eluted with *N* or 2 *N* NH_4OH using the ninhydrin test to indicate complete elution (*cf.*, Redfield, 1953). After removal of the excess ammonia by concentration *in vacuo*, the residue is dissolved in water and passed through a column of an anion exchange resin (Amberlite IRA-410, $CO_3^{=}$ cycle, etc.). Because the amino acids are ampholytes, they will be adsorbed on the anion resin whereas the cations will pass through. Arginine and sometimes lysine because of their basicity are, however, usually incompletely adsorbed on the anion resin. The amino acids are eluted with *N* HCl (Block and Bolling, 1951; Carsten, 1952ab; Brenner, 1951; Dreze, 1954; Mueller, G. C., 1955; Piez, 1952).

A column of Permutit 50 in the H^+ cycle, 16 mm. in diameter and 20-30 cm. high, is washed with 500-1000 ml. of 2 *N* HCl. The excess HCl is then removed by washing with 3-6 liters of water. The capacity of the column is ascertained by saturating it with *N* NH_4OH. The amino acid solution, containing salts, is dissolved in 1% HCl and passed through the column at 10-20 drops per minute. The column is washed with 200-300 ml. of water and the amino acids are eluted with *N* NH_4OH (Boulanger, 1951). Acid polypeptides which pass through Permutit 50 in the wash water may be adsorbed on a column of Deacidite (regenerated with 0.25 *N* sodium acetate) (*cf.*, Block, Durrum and Zweig, 1955).

2. Desalting in Special Solvents

The amino acid solution is adjusted to pH 4-5 with NH_4OH, filtered, concentrated to dryness, and ground to a fine powder. The powder is then suspended in 0.2 *N* *dl*-camphorsulfonic acid in acetone using 50 ml. of solution per gram of amino acid. After being stirred for at least one hour, the residue is removed by filtration and tested for undissolved amino acids with ninhydrin. If positive, it should be extracted with more reagent. The amino acid camphorsulfonates are decomposed with dry ammonia gas, and the insoluble ammonium camphorsulfonate is removed (McCollum, 1949, 1951, 1952; Rider, 1955), or the amino acids are precipitated by adding an excess of triethylamine. The triethylamine-*dl*-camphorsulfonate is soluble in acetone (Pollack, 1954).

An alternative procedure is to dry the serum, urine or other tissue fluid by lyophilyzation or on Whatman No. 3 filter paper. The paper is then cut into small pieces and the lipids are removed by several extractions with hot dichloroethane. After drying the paper at 60° C. *in vacuo,* the amino acids are extracted with a large volume (20 ml. per ml. of tissue fluid) of acetone containing one volume percent of concentrated hydrochloric acid or 1% of 14.8 *N* NH_4OH in methanol or ethanol (Boulanger, 1951; Lissitzky, 1954; Block, unpublished). The acid acetone solution should be prepared just before use. After extraction, the solvent is removed *in vacuo* and the residue is dissolved in 10% aqueous 2-propanol.

METHODS OF HYDROLYSIS OF PROTEINS

1. Acids

Hydrochloric, sulfuric, hydriodic, hydrochloric:formic and other acids have been used to hydrolyze proteins to their constituent amino acids. In general, the concentration of hydrolyzing acid used is inversely proportional to the time and temperature of the reaction.

Hydrochloric Acid: It is customary to boil the protein with from 2.5 to 5,000 times its weight of 6 N HCl under reflux for 18-24 hours. Obviously, when large quantities of protein are to be hydrolyzed, the expense of the acid and the difficulty of its removal after hydrolysis indicate the use of minimal quantities. However, when only small amounts of the protein are to be hydrolyzed for amino acid analysis, then it is advisable to use 6 N HCl equal to 1,000 to 5,000 times the weight of the protein. This large excess of hydrolyzing acid reduces the losses of amino acid.[*]

For many purposes, especially for analysis by microbiological methods, 3 N HCl at 15 lbs. pressure for 5-10 hours is also widely employed but it is not as satisfactory as 6 N HCl under reflux when crude proteins are to be analyzed.

It is well recognized (Block and Bolling, 1951; Schramm, 1950) that the rate of liberation of some amino acids from peptide linkage is faster than others; furthermore, it is known that the amount of destruction during hydrolysis increases with the length of heating. Thus, for the most accurate work, it is advisable to hydrolyze samples of the protein for different lengths of time, *e.g.*, 20, 40, 80 and 120 hours, and to determine the amino acid composition of each hydrolyzate. Then if it is observed that one or more amino acids (*e.g.*, cystine, methionine, serine, threonine) show a progressive decrease, then the true value may be assumed to be that found at zero time from a plot of the values obtained at different periods of hydrolysis. Likewise, if the quantity of an amino acid (*e.g.*, isoleucine, valine) gradually rises to a peak value, the assumed maximal value may be obtained graphically (Harfenist, 1953). These refinements are only valuable when the methods employed have the highest accuracy, and must be applied empirically for each separate protein. When investigating highly purified proteins it is also advisable to redistill the HCl several times in glass (Smith, E., 1954a; Sullivan, 1930).

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[*] Dustin (1953) claims that when 200 ml. of 6 N HCl are used to hydrolyze 35-30 mg. of protein the maximum loss of any amino acid amounts to only 3%.

Chromatographic assay methods require complete hydrolysis to amino acids for obvious reasons. Complete hydrolysis is also necessary when microbiological assay methods are employed because the growth response of many organisms is different (it may be greater or less) for the free amino acid than for the same amino acid in peptide linkage.

A point too often forgotten in many laboratories is that destruction of amino acids can take place even after the hydrolysis is ended, especially when the hydrolyzate contains free acid and humin. It is therefore advisable to remove the excess HCl as soon as possible after hydrolysis, filter off the humin,* decolorize with a small quantity of charcoal (Darco G-60), if necessary, and after evaporation to dryness, dissolve the amino acid hydrochlorides in 10% 2-propanol and store in the cold.

Hydrochloric Acid: Formic Acid. From 10 to 1,000 times the weight of protein is used of the following acid solutions; 38% HCl:88-90% HCOOH = 1:1 v/v or 6 *N* HCl:88-90% HCOOH = 1:1 v/v. This mixture has been claimed to be preferable to 6 *N* HCl for the analysis of cystine in insulin and nucleoproteins (Bakay, 1955). However, for the majority of proteins it is not superior to hydrochloric acid.

Hydrochloric Acid in the Presence of a Reducing Agent. Hlaziwetz (1873) added tin, $SnCl_2$ or Sn + $SnCl_2$, to the HCl before hydrolysis to reduce humin formation. The tin is then removed with H_2S. Sullivan (1937) uses 1 ml. of 20% $TiCl_3$ per 5 ml. of 20% HCl for hydrolysis. This greatly reduces humin formation. The excess titanium is removed by neutralizing to pH 6-7.

Hydriodic Acid. Kossel (1900) employed 5% HI or HI-HPO_2 for the hydrolysis of proteins; this prevents humin formation but converts methionine into homocysteine and cystine to cysteine. Its effect on serine and threonine awaits investigation. The carbohydrate reduction products may be removed from the HI hydrolyzate before complete hydrolysis of the protein (Holland, 1944). Hydrolysis with HI should be carried out in an inert atmosphere.

Sulfuric Acid. Although 8 *N* H_2SO_4 at atmospheric pressure is usually employed, other concentrations of acid and pressures may be used (Block and Bolling, 1951). The advantage of H_2SO_4 lies in the ease with which the excess acid can be removed with $Ba(OH)_2$. The humin is also largely precipitated along with the $BaSO_4$. H_2SO_4 is less effective, at equal concentrations, than HCl (Strocchi, 1954).

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*Horn (1953, 1955) has shown that the presence of humin in an hydrolyzate may actually increase the response of certain microorganisms to several amino acids. Rigby (1951) claims that an electric current passed through the hydrolyzate during heating will reduce humin formation. The humin may be removed by decolorization with charcoal or by precipitation with $Cu(OH)_2$ (Neuberg, 1950).

Other Acidic Reagents:

Aqueous Oxalic Acid. Used under pressure (Gilson, 1942).

Carbon Dioxide. Used under 50-60 atmospheres pressure and 250° C. (Karambelkar, 1950).

Aqueous Stannic or Titanous Chlorides. Used under pressure (Lieben, 1943).

20 N Sulfuric Acid:Glacial Acetic Acid:Water:Thiolactic Acid = 5:3:2:0.002 $^{v}/v$ (Sanger, 1954).

Sulfurous Acid. Used under pressure (Pedersen, 1952, 1953).

N Sulfuric Acid. Saturated with SO_2 under pressure (Pedersen, 1953).

5 N Formic Acid. This reagent is used to partially hydrolyze proteins. The peptides, etc., are precipitated by the Neuberg mercuric acetate reagent (*cf.*, Block and Bolling, 1951) after which the liberated peptides, etc., are completely hydrolyzed with HCl (Kofrányi, 1950).

Cation Exchange Resins. Used in the (H^+) cycle and treated with HCl or 0.296 N H_2SO_4 under pressure at 100-110° C. for 24-48 hours (Underwood, 1952a; Paulson, 1953a, 1953b, 1953c, 1954; Whitaker, 1955). The exact conditions vary with the type of protein.

Trifluoroacetic Acid, 80%. The excess acid may be removed by extraction with ether or other organic solvents (Block, Durrum and Zweig, 1955).

Hypophosphorus:Hydrochloric Acids. 10:90 $^{v}/v$ (Buck, 1954).

Dodecanesulfonic Acid:Hydrochloric Acid. Dodecanesulfonic acid, 0.01-0.1 N in 0.1-1.0 N HCl at 50° C. give hydrolyzates similar to peptic hydrolysis (Block and Bolling, 1951, p. 379; Schramm, 1948).

2. Bases

Sodium hydroxide, 5 N, or preferably 14% $^{w}/v$ barium hydroxide under reflux for 18-20 hours must be employed for liberating tryptophan from the protein. The excess barium ions are removed by neutralizing the diluted hydrolyzate with gaseous CO_2 or "dry ice."

Corfield (1955) uses 6.5 g. of $Sr(OH)_2 \cdot 8H_2O$ + 10.8 ml. of H_2O under reflux for 2-18 hours at 100° C. The excess strontium is precipitated with H_2SO_4. Yoritaka (1953a) claims that the addition of hydroxylamine to the alkali before hydrolysis decreases the destruction of tryptophan and tyrosine. The glass of the hydrolyzing vessel may also have an effect on the amino acids due to arsenic and iron (Gróh, 1951).

3. Enzymes

The use of proteolytic enzymes is not practical for the preparation of hydrolyzates for chromatographic methods and not advised for microbiological procedures (see above; also Block and Bolling, 1951). Sahyun (1949) has used proteolytic enzymes to separate protein from insoluble carbohydrates. The soluble peptides are then completely hydrolyzed with acid.

4. Losses During Hydrolysis

Arginine, Histidine and Lysine. Tristram (1939) has observed that the yield of arginine is decreased as the quantity of carbohydrate in the preparation hydrolyzed is increased. He has proposed the following empirical correction:

Losses of Arginine in the Presence of Carbohydrate

N in per cent	17.0	16.0	15.0	12.5-13.0
Correction factor	1.06	1.10	1.5	1.20

Block (1940a) and Knight (1942) have reported large losses of histidine during hydrolysis of yeast and tobacco mosaic virus if the carbohydrates and nucleic acids were not removed. Schein (1946) has observed that arginine is the most easily destroyed during hydrolysis with H_2SO_4; histidine is less easily destroyed but is readily racemized while lysine is the most stable of the diamino acids. However, boiling lysine with 20% HCl results in a 3% loss with the formation of aspartic acid, glycine, glutamic acid and α-amino adipic acid (Ishii, 1954).

Tyrosine. Jorpes (1932), in a careful study of the Millon-Folin method, found that 91 to 102% or a mean of 95.8 ± 0.9% (17 determinations) of free tyrosine could be recovered after heating at 100° C. for 14 to 18 hours with 5 *N* NaOH.

Lugg (1938) found that pure tyrosine is completely unaffected by heating for 20 to 30 hours at 100° C. with 7 *N* H_2SO_4, 5 *N* NaOH, or 5.5 *N* NaOH containing 5% $SnCl_2$. However, he showed that heating with 7 *N* H_2SO_4 in the presence of carbohydrate resulted in a considerable destruction of the amino acid.

Bolling (1943), as a result of 38 experiments on the recovery of tyrosine added to lactoglobulin (a protein which appears to be devoid of carbohydrate), and six experiments with tyrosine alone, reported a loss of from 11 to 17% of the added tyrosine after five-hour hydrolysis with 5 *N* NaOH in an oil bath at 115-125° C. She suggests a correction factor of 1.18, based upon an average recovery of 85%, to be applied with this protein and under these conditions of hydrolysis.

Tryptophan. The experiments of Homer (1915), of Gortner and his associates (*cf.*, Mitchell, 1929) and of Dawes (1949) showed that tryptophan is destroyed by hot mineral acids in the presence of $CuSO_4$, $Fe_2(SO_4)_3$, carbohydrates, aromatic aldehydes, and aliphatic aldehydes. However, in the absence of these mineral salts and aldehydes, tryptophan is very resistant to destruction by mineral acids (*cf.*, Sullivan, 1944; Monier, 1950). The findings of Homer, in 1915, on the stability of tryptophan to hot sulfuric acid have been confirmed by Hotchkiss (1941) who reported that only 0.7% of pure tryptophan is destroyed by refluxing under CO_2 with 6 *N* HCl for 24 hours.

The stability of tryptophan to hot alkalies, even in the presence of cupric or ferric ions and aldehyde groups, is considerably greater than to acids. Homer (1915) found that heating at 100° C. for 20 to 120 hours, with 14% baryta did not destroy tryptophan in commercial casein. On the other hand, Onslow (1921), Kraus (1925), and S. A. Gordon (1943) reported a considerable destruction of tryptophan by hot NaOH, Na_2CO_3, and $Ba(OH)_2$. Onslow (1921) noted that the quantity of tryptophan destroyed by hot baryta was less in the presence of other amino acids. Woolley (1945) claims that alkaline hydrolysis of proteins is unsatisfactory when tryptophan is to be determined by the microbiological method (*cf.*, Cuthbertson, 1951). Conversely, Greene (1944) reports that hydrolysis with 5 *N* $Ba(OH)_2$ for seven hours at 15 lbs. pressure is satisfactory for the microbiological determination of tryptophan. Greenhut (1946) advises a mixture of 50 mg. of pancreatin (Merck, U.S.P.) and 25 mg. of mucosa (hog, Wilson) at pH 8.2 per gram of protein at 37° C. for 24 hours for tryptophan assay.

As a result of Folin's experiments, hydrolysis with 5 *N* NaOH is generally employed. However, Jorpes (1932) recovered only 78 to 98% (mean, 82.4 ± 2.2%) of tryptophan added to gelatin after hydrolysis with 5 *N* NaOH at 100° C. for 20 hours. Webb (1945) determined the comparative effects of hydrolyzing for five hours at 125° C. (oil bath temperature) with 5 *N* LiOH, KOH, NaOH, 20% $Ba(OH)_2$ and $Ca(OH)_2$ (suspension). Casein, egg albumin, lactalbumin, gelatin and serum proteins were the test proteins. Recovery experiments with tryptophan were run. The results did not indicate the advantage of one alkali over another with the exception of $Ca(OH)_2$ which was definitely inferior.

Lugg (1938) has reported that the presence of 5% $SnCl_2$ in 5.5 *N* NaOH reduces the hydrolytic destruction of tryptophan in the presence of relatively large quantities of carbohydrate. When the protein is heated for 20 to 30 hours at 100° C. with 5 *N* NaOH, Lugg finds a 6% loss. If the protein is hydrolyzed under nitrogen with 5% $SnCl_2$ in 5.5 *N* NaOH, the loss is reduced to 3%. The use of $SnCl_2$ necessitates the removal of the tin with zinc, a tedious procedure. Yoritaka (1953a) used 0.5 g. of hydroxylamine for 100 ml. of 6 *N* NaOH in order to decrease the destruction of tryptophan during hydrolysis.

Bolling and Block (1943) found that varying quantities of tryptophan, added to lactoglobulin, were quantitatively recovered following hydrolysis with 5 *N* NaOH at 115 to 125° C. oil bath temperature. Both the Folin phenol (1919) and Millon-Lugg (Lugg, 1937) methods were employed and the analytical results were subjected to statistical analysis. However, this finding cannot be generalized as lactoglobulin is devoid or almost devoid of carbohydrate.

Phenylalanine. The chemical formula of this amino acid suggests its stability. It was, therefore, somewhat of a surprise when Block (1940b) found that casein, egg albumin, lactalbumin, gelatin and serum proteins gave more phenylalanine after hydrolysis with 5 *N* NaOH than with 8 *N* H_2SO_4, 20% HCl, HCl-HCOOH, and especially 57% HI. The slight superiority of NaOH hydrolysis over acid hydrolysis was confirmed by Fontaine (1942) and by Knight (1941). The latter investigators made the interesting observation that the presence of relatively large quantities of tryptophan (4% to 5%) in the protein results in high phenylalanine values by the chemical method (*cf.*, Block and Bolling, 1951). Therefore, the quantity of phenylalanine found must be corrected by subtracting the value corresponding to the amount of tryptophan present in the aliquot of the solution taken for analysis.

Bolling (1943) has confirmed the earlier findings that alkaline hydrolyzates (5 hours) may contain slightly more phenylalanine than acid hydrolyzates (18 hours). The addition of 2% and 4% of phenylalanine to lactoglobulin before acid hydrolysis gave 95% and 92% recoveries. However, these losses were not statistically significant (critical ratios less than 3.3). The addition of 12% of the weight of the protein as phenylalanine resulted in only an 87% recovery. This loss was significant (critical ratio 8.1). On the other hand, Bolling's experiments (1944) indicated that when phenylalanine is heated at 110 to 115° C. for five hours with 5 *N* NaOH it is destroyed or so changed as not to permit estimation by the Kapeller-Adler method. The amount of destruction increases as the percentage of phenylalanine in terms of protein hydrolyzed is increased. Thus, when 2% of phenylalanine was added to lactoglobulin before hydrolysis, approximately 90% was recovered; 4% of phenylalanine gave a recovery of approximately 80%, while the addition of 12% of phenylalanine resulted in the recovery of only 70%. In the absence of protein the loss was much greater. When 4 mg. of phenylalanine were heated at 110 to 115° C. (oil bath temperature) with 8 ml. of 5 *N* NaOH for five hours, only 25% of the phenylalanine could be found. Foster (1945) also reported that some phenylalanine is destroyed during hydrolysis with 6 *N* HCl.

Comment. It is apparent from the above discussion that destruction of tyrosine, tryptophan and phenylalanine can and often does occur during hydrolysis. The amount of destruction is dependent upon the conditions; this includes strength of acid or alkali, time and temperature of hydrolysis, presence or absence of oxygen and oxygen carriers, presence of aldehydes, and the ratio of the amino acid in question to undecomposed protein and to the liberated amino acids.

Cystine and Cysteine. The destructive effects of alkali on cystine and cysteine are so extensive as to preclude the use of these reagents as hydrolyzing agents of proteins when either of these two amino acids *per se* are to be determined.

The effects of acid hydrolyzing agents on cystine and cysteine are variable. Bolling (1943) found no loss of cystine as indicated by recovery experiments during 20-hour hydrolysis of β-lactoglobulin with 10 volumes of a 1:1 mixture of 18% hydrochloric acid and 90% formic acid. The nonspecific Folin phospho-18-tungstic acid and the specific Fleming-Vassel *p*-phenylenediamine methods were used to determine cystine.

Lugg (1933), using a modification of the Folin method, likewise found very slight losses of cystine and only a small loss of cysteine during HCl hydrolysis in the absence of carbohydrate. In the presence of small amounts of carbohydrate, 6% to 7% cystine was lost while 85% to 100% of the cysteine could not be recovered. When relatively large quantities of carbohydrate were present even cystine was largely destroyed. Halver (1946) says that, although carbohydrates cause destruction of cystine during hydrolysis, some cysteine is lost during the hydrolysis of carbohydrate-free proteins. He believes that cysteine determinations based on any method involving preliminary acid hydrolysis of the protein is of doubtful significance. Yoritaka (1954) reports that HCl hydrolysis of cystine yields alanine and serine and if carbohydrate is present, glycine is also formed.

Bailey (1937) using modifications of the Folin and Sullivan cystine methods, found that cystine added to edestin gave 99 ± 2% recoveries, but the *absolute* cystine values varied, indicating that part of the cystine in peptide linkage may be more readily destroyed. He reports that 30% of the cystine and 20% of the methionine present could be lost on hydrolysis with 5 *N* HCl for 15 hours.

G. L. Miller (1937) found that when insulin, a protein devoid of carbohydrate, was hydrolyzed with an equal mixture of concentrated HCl and formic acid for 48 hours there was little or no destruction of cystine. If the hydrolysis was carried out for the same length of time with 20% HCl alone, an appreciable loss was observed. It should be pointed out however, that 6 to 8 hour hydrolyses with 18 to 20% HCl are entirely adequate for all protein preparations which we have investigated.*

Lugg (1938) refluxed the protein with an excess of 57% HI under nitrogen for 6 to 8 hours. The hydrolytic losses are minimized in this strongly reducing atmosphere. Except for technical disadvantages, hydriodic acid appears to be the most suitable hydrolyzing agent for use with protein preparations which contain carbohydrate and similar compounds. It is usually unnecessary in the determination of cystine in protein preparations of adequate nitrogen content (*cf.*, Springer, 1952, 1953).

Probably the most careful series of investigations on the hydrolytic destruction of cystine are those of Sullivan, Hess and their collaborators. Sullivan (1939) found somewhat less cystine in insulin hydrolyzates after hydrolysis with 20% hydrochloric acid than after heating with 20% HCl in 90% formic acid for 12 hours (oil bath, 125-135° C.). They stress the point that if hydrolyzates are kept for some time there is a significant loss of cystine as estimated by Brdicka's polarographic method or by Sullivan's colorimetric procedure.

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*Lyman (1955) found that the loss of cystine during HCl hydrolysis is reduced if a relatively large quantity of tyrosine is added to the protein and the air is replaced by hydrogen sulfide.

Hess (1941) investigated the hydrolytic losses of cystine in a carbohydrate containing protein (tobacco mosaic virus). The Sullivan colorimetric and the Mörner-Okuda iodine titration techniques for estimating cystine were used. Their results are summarized in Table I.

TABLE I

Hydrolyzing Agent	*Time of Heating Hours*	*Per Cent of Cystine*	
		Colorimetric	*Titrimetric*
20% HCl	6	0.47	0.48
20% HCl in Formic Acid	24	0.53	0.48
6 *N* H_2SO_4 under N_2	12	0.67	0.71
20% HCl plus 1% $TiCl_2$	2	0.64	0.64
Concentrated HI-HCOOH under N_2	24	0.69	0.69

Methionine. Bailey (1937) reported that 20% of the methionine present in a protein can be lost during hydrolysis in the presence of large quantities of carbohydrate while Osono (1955) has found that refluxing methionine in 20% HCl results in the formation of some homocystine, homocysteine, and glycine.

Serine and Threonine. Serine and threonine, like cystine, are readily destroyed by boiling with dilute alkalies, but appear to be quite stable to hydrolysis with dilute acids, even in the presence of carbohydrates. However, Borchers (1942) found that long heating and high concentrations of sulfuric acid would also result in the destruction of threonine. Our own experience has suggested that the quantity of threonine found in a protein hydrolyzate is somewhat dependent on the time and temperature of hydrolysis.

Nicolet (1942) has reported that the phosphorylated serine groups in casein, vitellin, etc., undergo destruction during acid hydrolysis. He, therefore, suggests warming the phosphoprotein at 37° C. for 24 hours with an excess of 0.25 *N* NaOH to liberate the phosphoric acid groups and then hydrolyze with acid as usual. "To serine as determined in this hydrolyzate is added a (small) correction for non-phosphorylated serine destroyed by alkali, and one molecular equivalent for all phosphate liberated." This correction results in considerably higher serine values than would be obtained directly. Thus, vitellin (N = 15.26%) yielded 7.70% serine, uncorrected, 9.0% corrected; while casein (N = 15.80%) gave 5.5% serine, corrected 7.4%.

Rees (1946) and Hirs (1954a) showed that serine and threonine are increasingly destroyed during hydrolysis with HCl. Rees' corrections for a 5% loss of threonine and a 10% loss of serine during 20-hour hydrolysis have been widely employed. This is probably not justified as no two protein preparations may be expected to behave identically under different laboratory conditions, *i.e.*, purity of acid, temperature, ratio of acid to protein, etc. Corfield (1953) showed that even in a simple protein like salmine there was a 14% loss of serine on boiling with 5.5 *N* HCl for 24 hours.

5. Estimation of the Completeness of Hydrolysis

The liberation of carboxyl and amino groups from peptide linkage is the object of protein hydrolysis. Hydrolysis is considered complete when a maximum number of $-COOH$ and $-NH_2$ groups have been liberated. The carboxyl groups can be readily estimated by one of the modifications of the Schiff-Sørensen formol titration method or by oxidation with ninhydrin.* (*Cf.*, Block and Bolling, 1951; MacFadyen, 1950ab; Smith, A. M., 1951a for mechanism.) The amino nitrogen can be determined by the Van Slyke method (*cf.*, Irving, 1944a, 1944b; Schmidt, 1938).

Titration of Amino Groups With Copper. Amino acids form soluble copper salts. The copper content and consequently the amino nitrogen of the filtrate is determined iodometrically. This reactions forms the basis of the procedure for the determination of amino nitrogen developed by Pope and Stevens (1939) and modified by Block and Bolling (1951) as given below. (See also Baudet, 1955; Beauchene, 1955; Schroeder, 1950a and Spies, 1951a, 1952.)

Reagents

Cupric chloride. Dilute 27.3 g. $CuCl_2 \cdot 2H_2O$ to 1000 ml.

Tri-sodium phosphate. Dilute 64.5 g. $Na_3PO_4 \cdot 12H_2O$ to 1000 ml.

Borate buffer. Dissolve 57.2 g. $Na_2B_4O_7 \cdot 10H_2O$ in 1500 ml. H_2O. Add 100 ml. of *N* HCl and dilute to 2000 ml.

Cupric phosphate suspension. Mix 1 volume of $CuCl_2$ and 2

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*Peri-naphthindan-2,3,4-trione hydrate may be substituted for ninhydrin (Moubasher, 1949a; Schoenberg, 1949).

volumes of Na_3PO_4, then add 2 volumes of borate buffer in that order. Prepare the mixture as needed.
Thymolphthalein. Dissolve 250 mg. in 100 ml. of 50% ethanol.
Sodium thiosulfate. Dilute $Na_2S_2O_3$ (Anachemia, Ltd.) to 0.01 *N* with 0.1% sodium borate as needed.
Starch. Prepare 0.5% solution of starch in 20% NaCl, boil, and let stand over-night. Store in cold.

Method: Pipette a sample (10 to 20 mg. of total nitrogen) into a 50-ml. graduated cylinder. Dilute to 15 ml. Add 4 drops of indicator and sufficient *N* NaOH to produce a blue color. Then add 30 ml. of copper suspension, dilute to 50 ml. and mix. Filter immediately through soft paper. Refilter first 5 ml. of filtrate. Remove a 5-20 ml. aliquot, add H_2O to 20 ml., then add 0.5 ml. of glacial acetic acid and 2 g. of KI. Titrate with 0.01 *N* $Na_2S_2O_3$ at a rapid rate until the solution is pale yellow, then add 1-2 ml. of starch indicator, and titrate the solution with swirling after each drop to one drop beyond colorless.

Reaction of Amino Acids With Ninhydrin. Ammonia resulting from the oxidative deamination of amino acids with ninhydrin is aerated or steam distilled* into boric acid and titrated (Sobel, 1945).

Reagents

Caprylic alcohol. Saturate with thymol.
Ninhydrin (solid).
Citrate buffer, pH 2.5. Grind 2.06 g. of trisodium citrate and 19.15 g. of citric acid to a fine powder.
Hydrogen peroxide, 30%.
Potassium hydroxide, saturated. To a cylinder containing water under mineral oil 1 inch thick, add solid KOH to saturation. Preserve under oil.
Indicator. Add 10 parts of 0.1% bromocresol green to 1 or 2 parts of 0.1% methyl red in 95% ethanol.
Boric acid. Dilute 20 g. of H_3BO_3 to 1 liter. Add 20 ml. of indicator per liter.
Hydrochloric acid. Standard 0.0714 *N* HCl.

Method: Add 1 ml. of solution containing 20-100 mg. of carboxyl nitrogen to an aeration tube containing 0.3 mg. of buffer and 50 mg.

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*The Conway microdiffusion method may also be used (McConnell, 1952).

of ninhydrin. The pH should be 2.4-2.6. Shake to mix and place in boiling water for 10 minutes. At the end of two minutes heating, shake to dissolve the ninhydrin. After 10 minutes heating, add 3 drops of 30% H_2O_2, shake and heat for three minutes longer. Set up the tubes for aeration or distillation. Add 1 ml. of saturated KOH and aerate into H_3BO_3 for 40 minutes using 1.5 ml. of 2% boric acid to trap the ammonia. Titrate* with HCl or $KHIO_3$ (*cf.* Eades, 1955). Calculate as follows:

$$\text{ml. of } 0.0714\ N\ \text{HCl} \times 1000 = \text{mg. of amino acid N}$$

Comment: Schoenberg (1949) and Moubasher (1949a, 1949b) substituted peri-naphthindan-2,3,4-trione hydrate for ninhydrin. This reagent is recoverable.

✓ *Colorimetric Estimation of Amino Groups.* The following is the method of Harding (1916). To 1 ml. of neutralized unknown (0.01-0.08 mg. of amino nitrogen) add 1 ml. of 10% aqueous pyridine and 1 ml. of 2% aqueous ninhydrin. Stopper the test tube lightly with a cotton plug and place in a boiling water bath for 20 minutes. Cool in water, dilute to 50 ml. and read at 570 mμ. Leucine may be used to prepare the standard curve (Green, M., 1955).

Comment: This excellent method has been largely forgotten. It is quite obvious that any of the other conditions for carrying out the ninhydrin reaction as given elsewhere in this book or in Block, Durrum and Zweig (1955) may also be employed. A suggested procedure is that of Cocking (1954).

Mix 1 ml. of an amino acid solution containing 0.05-2.8γ amino nitrogen with 0.5 ml. citrate buffer of pH 5 (0.2 *M*). Add ninhydrin in methyl cellosolve (0.2 ml., 5% ^{w}/v) and KCN in methyl cellosolve (1 ml., 2% ^{v}/v) to this solution either separately or as a single solution. Separately these reagents are stable for at least 1 month, and mixed, for at least 1 week. Heat the well-mixed solution for at least 15 minutes at 100° C. and cool for 5 minutes in running tap water. The b.p. of the water:methyl cellosolve mixture is greater than 100° C. and using tubes stoppered with a glass marble, evaporation losses during the heating period are negligible. Make the solution up to a convenient volume with ethanol (60% ^{v}/v) and determine the optical density at 570 mμ. The colors are quite stable for at least 1 hour at room temperature. Most of the common amino acids give colors equivalent to 100 ± 1% of that of pure dioxohydrindylidene-dioxohydrindamine (DYDA) except tryptophan (80%) and lysine (110%). Ammonia reacts yielding a color equivalent to only 33% of that of pure DYDA.

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*The quantity of ammonia may be also estimated colorimetrically using ninhydrin (Laki, 1954).

The Folin sodium-2-naphthoquinone-4-sulfonate method is not recommended for amino acid mixtures (Fusman, 1950). Zimmerman (1952) employed 1% 1,3,5-trinitrobenzene in 20% dioxane to determine amino acids colorimetrically.

Spectrophotometric Determinations of Amino Groups. The method is that of Fraser (1953b) and consists of the following procedure. One ml. of 1% sulfanilic acid in 10% HCl is mixed with 1 ml. of 5% aqueous $NaNO_2$ and added to 0-10 ml. of amino acid solution. This mixture is allowed to stand at room temperature for 30 minutes, after which time 3 ml. of 20% Na_2CO_3 is added. This solution is then diluted to 25 ml. with water and read at 363 mμ. The quantity of color is proportional to the free amino groups.

Ultraviolet Spectrophotometric Method for the Estimation of Amino Groups. This procedure was developed by Spies (1951a, 1952) and requires the use of a Beckman quartz spectrophotometer Model DU with 10 mm. quartz cuvettes.

Reagents

Copper chloride. 8.52 g. $CuCl_2 \cdot 2H_2O$ per liter of water (0.05 *M*).

Sodium borate buffer, pH 9.1-9.2. Dissolve 40.3 g. of Na_4BO_7 in 4 l. H_2O. Filter. Just before use dissolve 6.0 g. NaCl in 100 ml. of borate solution.

Method: To 5.0 ml. of NaCl-borate buffer in a 25-ml. glass stoppered Erlenmeyer, add 5.0 ml. of aqueous amino acid solution. Then add 0.1 ml. of $CuCl_2$ solution, shake and allow to stand at room temperature for 10 minutes. Centrifuge in a closed 12-ml. tube for 5 minutes. Decant *clear* supernatant liquid. Read against a water blank at 230 mμ. Alanine is used as the standard. The standard curve is prepared by plotting the log of the percent transmission against the concentration.

Polarographic Determination of Amino Acids. In a procedure developed by Norton (1954) individual amino acids can be determined polarographically with an error of ± 2% in borate buffer in the presence of phthalaldehyde. This method, however, cannot be used in mixtures of amino acids or in the presence of substances which react with the aldehyde.

6. Determination of End Groups

The use of specific reagents for the qualitative identification of the amino and carboxyl ends of peptides and proteins has been in the literature for many years. However, the introduction by Sanger (1945; *cf.*, 1951, 1953) of dinitrofluorobenzene for the determination of the amino end groups has permitted remarkable advances in the elucidation of the structure of proteins and peptides and has stimulated research for other methods of end group determination as well as methods for the stepwise degradation of polypeptides. Although this work is outside the scope of this book, some of the important references in this field are: Akabori, 1954; *cf.*, Block, Durrum and Zweig, 1955; Dickman, 1952; Fraenkel-Conrat, 1954, Fromageot, 1950b, Fox, 1951, Ingram, 1950; Leonis, 1954ab; Reith, 1954; and Sanger, 1945, 1951, 1953.

7. General Comments

Although methods for the preparation of the protein sample for analysis have been mentioned above the general principles are obviously simple. The protein must be prepared as devoid as possible from contaminating substances, especially carbohydrates and fats.

Heat coagulation is often employed to separate soluble proteins from inorganic salts, etc. It is usually assumed that the amino acid composition of the heat coagulated protein is the same as that of the original substance. However, Calvery (1936) has shown that the amino acid composition of pepsin and of the heat coagulum obtained from it are not identical. Kiesel (1936) has reported similar results with heat coagulated edestin and the undenatured protein. The importance of these experiments is obvious.

It is often difficult to ascertain the quantity of soluble protein present in a crude solution from the nitrogen content alone, therefore the following procedure is often valuable: Place 2 ml. of protein solution in a centrifuge tube, add 2 ml. of 20% trichloroacetic acid, and heat to 90° C. in a water bath for 5 minutes. Centrifuge and wash the precipitate 3 times with 10% trichloroacetic acid. Dry the precipitate to constant weight at 110° C. (Hoch, 1953).

Chibnall (1943) has pointed out that certain proteins are so hygroscopic when anhydrous that they cannot be handled without special precautions; moistures and nitrogen contents should therefore be determined on separate samples of the air-dried protein.

CHAPTER III

Chemical Methods for the Determination of Amino Acids

THE MANY chemical methods for the determination of the amino acids have been summarized previously (Block and Bolling, 1951) and will not be given in this book. However, we will attempt in this Chapter to describe briefly some of the chemical methods which have appeared since our earlier publication.

BASIC AMINO ACIDS

1. Isolation of Arginine, Histidine, and Lysine

This procedure is that of Robson (1952; 1953). After hydrolysis of 25 g. of gelatin with 8 N H_2SO_4 and removal of the excess acid (to pH 2.5) with $Ca(OH)_2$, the aromatic acids are removed on a charcoal column. The nonaromatic amino acids are eluted with 0.2 N H_2SO_4. The effluent and sulfuric acid washings are combined and the H_2SO_4 is removed with $Ba(OH)_2$. Then 20 g. of picric acid are added and the solution is warmed to dissolve the precipitate. The picrates of the basic amino acids are adsorbed on a 100-gram carbon column. The nonbasic amino acid picrates are removed by washing with water. The basic amino acid picrates are obtained as follows: a) lysine and hydroxylysine by elution with 0.5 N H_2SO_4; b) histidine after lysine by elution with 0.5 N H_2SO_4 and by N H_2SO_4; and c) arginine is separated from the carbon by boiling the extruded column (lowest quarter) with N H_2SO_4.

2. Arginine and Lysine

Arginine and lysine are separated from the other amino acids by exchange on Decalso (Bernardi, 1950; *cf.*, Block and Bolling, 1951; Šorm, 1950). After washing the column to remove other amino acids, arginine and lysine are eluted with 5% NaCl. The elutriate is concentrated *in vacuo* and the NaCl is precipitated by the addition of an equal volume of methanol to the hot solution. On cooling, the lysine hydrochloride precipitates. Arginine is obtained from the filtrate after removal of the methanol and adjustment to pH 10.5-13.0 with NaOH (*cf.*, Schramm, 1947).

A cation exchange resin in the H^+ cycle is preferable to Decalso. The aromatic amino acids may first be removed by adsorption onto charcoal and the neutral amino acids and histidine eluted from the column with pyridine. The lysine and arginine are then eluted with *N* NH_4OH (Block, 1946; Kunin, 1951; Merck & Co., 1953; Rauen, 1948; Roberts, E. J., 1952).

3. Arginine and Lysine by Precipitation With Copper

Arginine copper and lysine copper, prepared from the hydrochlorides by boiling with basic cupric carbonate, are precipitated quantitatively as the HgI_4 complexes (Taurins, 1950). The complexing agent, HgI_4, is prepared from 6.65 g. KI + 9.09 g. HgI_2 in 100 ml. H_2O.

4. Arginine Flavianate

The direct precipitation of arginine flavianate from protein hydrolyzates (*cf.*, Block and Bolling, 1951, p. 44) is contaminated with histidine and lysine (Heathcote, 1950a).

5. Arginine and Other Guanidino Compounds

Eastoe (1954a) separated the guanidino compounds resulting from treating proteins with *O*-methylisourea on Dowex-50 with 0.1 *M* phosphate buffer (pH 6.75) and with 0.2 *M* citrate buffer (pH 6.50). (See also Chapter VI.)

6. Arginine and Other Guanidino Compounds (Sakaguchi)

The widely used Sakaguchi test (*cf.*, Block and Bolling, 1951, p. 47 *et seq.*) for the unsubstituted guanidino group has not given too satisfactory quantitative results. Some of the modifications introduced since our previous monograph are given below:

a. Sakaguchi Method According to Salo (1949). This procedure is as follows: *x* ml. of hydrolyzate + 5-*x* ml. of H_2O + 1 ml. 10% NaOH + 1 ml. of 0.1% α-naphthol. Mix and cool in running water for 5 minutes. Add 1 ml. of diluted NaOCl (proper dilution estimated by trial and error) with a rapid delivery pipette. Read color in the colorimeter. Using three different sized aliquots of the hydrolyzate, plot the apparent arginine concentration *vs.* the concentration of the protein. The true arginine value is obtained by extrapolation to zero hydrolyzate (*cf.*, Block and Bolling, 1951, p. 47-50).

b. Sakaguchi Method According to Ved Vrat (1952). NaOBr is used in place of NaOCl, being made from 2 g. of Br_2 in 100 ml. of

5% NaOH. Excess reagent is destroyed with 40% urea (*cf.*, Sakaguchi, 1951; Gómez, 1953; Strocchi, 1954). Van Pilsum (1953) uses sodium thiosulfate to destroy the excess oxidizing agent.

c. Sakaguchi's New Method (1948). Add to 5 ml. of the test solution, 1 ml. of 0.02% 8-hydroxyquinoline* in 1 ml. of 10% NaOH. Cool in an ice bath 2-3 minutes. Add the optimal quantity of NaOBr (2 g. Br_2 in 100 ml. of 5% NaOH) estimated by trial and error. Shake, wait 20 sec. and add 1 ml. of 40% urea. Mix and read within 5 min. with 550 mμ filter.

7. Histidine by the Knoop-Kapeller-Adler Reaction (*cf.*, Block and Bolling, 1951, p. 59 *et seq.*)

a. Hunter's Modification (1952; 1955)

Reagents

H_2SO_4. 1.9-2.0 *N*

Bromine. Dissolve 0.5 ml. of Br_2 in 50 ml. of *tert*-butanol + 50 ml. of 0.6% KBr. Store in dark at 4-5° C. Do not keep over 4 weeks.

Arsenite. Dissolve 3 g. As_2O_3 in 10 ml. of 10% NaOH and dilute to 100 ml.

Copper acetate. To 90 ml. of H_2O add 2.1 ml. of glacial acetic acid, 0.26 ml. of 1% $CuSO_4 \cdot 5H_2O$ and dilute to 100 ml. Then add 100 g. of $CH_3COONa \cdot 3H_2O$. Volume = 172 ml. containing 9.6 γ $CuSO_4$ per ml.

Method: To 5 ml. of solution containing between 0.12-0.28 γ of histidine, add 0.5 ml. of 2 *N* H_2SO_4. Mix, warm to 25° C. and in a dark room add 0.25 ml. of bromine reagent. Stopper and place in dark at 25° C. for 2.5 hours. Add 5-6 drops (0.25 ml.) of arsenite reagent, then add rapidly with shaking 1.2 ml. of acetate reagent. After 14 minutes at 25 ± 0.2° C., dilute with water at 10° C. to 10 ml., and read with 540 mμ filter against reagent blank. The red pigment adsorbs on the glass and requires HNO_3 to clean.

Comment: Other modifications of the Knoop reaction are given by Kapeller-Adler (1949), Negelein (1952), Oyamada (1944), Reifer (1953) and Tsuverkalov (1951). (See also Block and Bolling, 1951;

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*Kraut (1951) employs 1-naphthol-8-sulfonic acid in place of 8-hydroxyquinoline, while Janus (1956) uses 7-chloro-8-hydroxyquinoline.

p. 59 *et seq.*) Cowgill (1955) has developed a new colorimetric method for histidine based on *p*-dinitrobenzoyl chloride splitting of the imidazole ring.

8. Lysine and Hydroxylysine Isolation as Benzylidene Copper

The lysine solution is treated with an excess of $Cu_3(PO_4)_2$ suspension at pH 8.8 (Turba, 1948; Ackermann, 1951). The solution is filtered and an equal volume of methanol is added to the filtrate. An excess of benzaldehyde is shaken with the solution and the pH adjusted to 8.8. Copper benzylidene lysine precipitates and is washed with a mixture of water, benzene and methanol at pH 8.8 (*cf.*, Ramachandran, 1952). After removal of the reagents the amount of hydroxylysine can be estimated by oxidation with periodate (*cf.*, Block and Bolling, 1951, p. 69; Ramachandran, 1953).

9. Lysine and Histidine Isolation as the Benzenesulfonates

The mineral acid is removed from a protein hydrolyzate with an anion exchange resin and the solution evaporated to dryness (McCollum, 1951; 1954). The amino acids are suspended in 10-100 volumes of 0.5 *N* benzenesulfonic acid in acetone at 8-10° C. Dilute the solution with 4 volumes of acetone and allow to stand in the cold for 2-4 hours. Filter off the lysine and histidine benzenesulfonates. Lysine benzenesulfonate is insoluble in 0.25 *N* trichloroacetic acid in acetone, while histidine benzenesulfonate is soluble (*cf.*, Pollack, 1954; Wierzchowski, 1951).

Comments: Marenzi (1953) has modified the Nelson-McFarlane colorimetric method for lysine (*cf.*, Block and Bolling, 1951, p. 64). Emmick (1951a) described the racemization of D-lysine by heating on a cation exchange column in the ammonia cycle. He also reported the resolution of DL-lysine with L-glutamic acid. L (+)-lysine glutamate precipitates from aqueous methanol.

Hamilton (1954a, 1954b) has shown that although ornithine is not naturally present in collagen it may be produced during "liming." He has separated the basic amino acids, including ornithine, from each other by ion exchange chromatography (see Chapter VI). Morrison (1952), Zacharias (1952) and Virtanen (1954) have reported the isolation of pipecolic acid and hydroxypipecolic acids in plants. These are derived from lysine and hydroxylysine, respectively. Van Tamelen (1953) synthesized isolysine (β-γ-diamino-*n*-caproic acid).

AROMATIC AMINO ACIDS

1. Chromatographic Separation of Tyrosine, Tryptophan and Phenylalanine

This is the procedure of Hall (1951).

Reagents

Buffer, pH 9.7. 0.1 *M* $Na_2CO_3/NaHCO_3$.
Developer. 1% *n*-butanol in buffer.
Eluant *A*. 1% 1, 1-Dimethylpropanol in buffer.
Eluant *B*. 1% 4-methyl-1-pentanol (isoamyl alcohol) in buffer.
Eluant *C*. 1% Benzyl alcohol in buffer.
Adsorbant. Super-Cel:Carboraffin = 3.1 $^w/w$. Both should be purified with HCl and NH_4OH.

Method: The aromatic amino acids in buffer are adsorbed on the carbon, developed with aqueous butanol. Tyrosine is eluted with *A*, phenyalanine with *B*, and tryptophan with *C*. Recovery = 96%.

2. Determination of Tyrosine with 1-Nitroso-2-Naphthol (Gerngross Method, 1933)

a. Udenfriend's (1952) Method (*cf.*, Block and Bolling, 1951, p. 116)

Reagents

A. 1-Nitroso-2-Naphthol, 1% in 95% ethanol
B. $HNO_3:H_2O = 1:5$ $^v/v$ containing 0.5 mg. of $NaNO_2$ per ml.

Method: To 2 ml. of protein-free filtrate (containing 0.03-0.80 γ of tyrosine or tyramine) add 1 ml. of *A* and 1 ml. of *B*. Stopper, heat at 55° C. for 30 minutes, cool and extract with 10 ml. of dichloroethane ($ClCH_2CH_2Cl$). Centrifuge, remove water layer, and read color with 450 mμ filter.

b. Ramachandran's Method (1951)

Dilute tyrosine solution to 7.1 ml., add 2 ml. of formaldehyde, 0.5 ml. of 0.1% 1-nitroso-2-naphthol, and 0.4 ml. of concentrated HNO_2. Mix after each addition. Heat in water bath at 80° C. for 2 minutes. Cool rapidly to room temperature and read color at 490 mμ.

Comment: Giral (1948) has used the Gerngross method for tyrosine, as well as the blue color formed by this amino acid with 0.5% selenium oxide in H_2SO_4, for its quantitative determination. Rusconi (1951) and Monnier (1953) have determined tyrosine polarographically in 0.15 *N* HNO_3 while Brodskii (1954) has used ultraviolet adsorption before and after treatment with 0.2 *N* nitrous acid.

3. Determination of 3,5-Diiodotyrosine

This is the procedure of Moudgal (1954); see also Block and Bolling (1951), p. 141.

Dissolve the sample in 4.5 ml. of water and add 0.5 ml. of *M* NaCl. Add 0.2 ml. of *N* HCl and 0.2 ml. of 1% $NaNO_2$. Warm at 38° C. for 30 minutes. Add 0.05 ml. of 1% $CoCl_2$ and 5 ml. of saturated sodium acetate:20% sodium acetate = 3:2 $^v/v$. Read using a blue filter. Error = ± 3%.

4. Determination of Diiodotyrosine, Triiodothyronine, Thyroxine and Other Similar Compounds

Reagents

0.05 *N* ceric ammonium sulfate in 2 N H_2SO_4
0.10 *N* sodium arsenate (neutral or slightly acid)

Method: The iodinated amino acids are separated by paper chromatography (Whatman No. 1 or No. 3) with 2-butanol:ammonia* = 3:1 $^v/v$ (Block and Bolling, 1951) in the presence of 100 ml. of conc. NH_4OH, or with CH_3OH:0.2 *N* CH_3COONH_4 (pH 6.1) = 10:25 followed by 2-butanol:NH_4OH *in the same direction* but stopping the solvent 1 cm. below the diiodotyrosine spot or band. This procedure separates thyroxine, triiodothyronine, diiodothyronine, diiodotyrosine, monoiodotyrosine and inorganic iodide. Develop twice. The chromatogram as sprayed with a 3:2 mixture of 10% $Ce(HSO_4)_4$ in 10% $^w/v$ H_2SO_4 and 5% $NaAsO_2$ or the appropriate iodide spots are excised, the organic iodide is reduced to inorganic iodide and the quantity of iodine is determined colorimetrically using the ceric sulfate-arsenous acid reaction which is catalyzed by I^- (Feigl, 1954; Long, 1955).

$$2\ Ce^{4+} + AsO_3^{3-} + H_2O \longrightarrow 2\ Ce^{3+} + AsO_4^{3-} + 2\ H^+$$

The quantity of iodide present is measured by the disappearance of the yellow color of the ceric ions (*cf.*, Bowden, 1955; Lissitsky, 1954b, 1955).

Comment: Diiotyrosine, monoiodotyrosine, triiodothyronine and thyroxine have been determined radiologically by Gleason (1955), Gross (1954), Larson (1952), Reinecke (1954), Roche (1951c), Tong (1954) and others (*cf.*, Block, Durrum and Zweig, 1955). Taurog

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*28%.

1955) suggests the addition of 7×10^{-4} *M* of thiouracil to the sample to reduce formation of artifacts during paper chromatography. Thyroxine and triiodothyronine have also been estimated polarographically (Borrows, 1949 and Evert, 1954).

5. Determination of Tryptophan

Many procedures for the qualitative and quantitative assay of tryptophan and other indoles have been described in detail in Block and Bolling, 1951, p. 117 *et seq.* Some newer modifications have been reported of the well-known Tiedemann and Gmelin (1825) chlorine-water and the Kühne (1875) bromine-water tests for indoles in which the colored reaction products are dissolved in amyl alcohol (Kisch, 1950) or chloroform (Sekine, 1950; *cf.*, Shimada, 1941). Tryptophan gives a color with 1-(-diethylaminoethylamino)-naphthalene oxalate when diazotized in the presence of ammonium sulfamate (Kawamata, 1953). Spies (1949) employed basic lead acetate in the alkaline hydrolysis of proteins to protect the tryptophan from the destructive action of cystine and its decomposition products. Giral (1950), Obata (1952), Portner (1953), Spies (1950a, 1950b) and Steers (1949) have described modifications of the Voisenet-Rhode *p*-dimethylaminobenzaldehyde method for tryptophan (*cf.*, Block and Bolling, 1951, p. 119) and Dumazert (1954) has modified the Hopkins-Cole reaction. M. Gordon (1949) and Besso (1952) described fluorometric and polarographic procedures for this amino acid. D. L. Morris (1955) has separated tryptophan on an anion exchange resin (Amberlite IRA-400, regenerated with 4% NaOH) from the other amino acids in a 20% NaOH hydrolyzate. The tryptophan is eluted with 6% acetic acid.

6. Determination of Phenylalanine

The mechanism of the Kapeller-Adler reaction for phenylalanine (*cf.*, Block and Bolling, 1951, p. 136 *et seq.*) has been investigated by Beer (1951) and modified by Zalta (1952).

Reduction of Phenylalanine to Phenylethylamine (*Udenfriend, 1953*). To 2 ml. of a solution containing 0.03-0.30 γ of L-phenylalanine in a 60-ml. glass stoppered bottle, add 0.5 ml. of 0.7 *M* citrate buffer of pH 5.5, 0.5 ml. of a *Streptococcus faecalis* suspension, and incubate 2 hours at 37° C. Cool, add 0.3 ml. of 10 N NaOH + 15 ml. of chloroform:isoamyl alcohol = 97.6:3.3 v/v, shake 5 minutes, and centrifuge 2 minutes. Put solvent layer in a 15-ml. glass-stoppered bottle, add 0.5 ml. of a 5.0% aqueous solution of methyl orange and dilute with an equal volume of saturated boric acid *just* before use. Shake 2 minutes, centrifuge and remove excess methyl orange with a pipette. Put 10 ml. of solution into a colorimeter tube and add 2 ml. of alcoholic H_2SO_4 (2 ml. of conc. H_2SO_4 in 100 ml. of absolute ethanol). Mix and read at 540 mμ.

THE SULFUR-CONTAINING AMINO ACIDS

1. Cystine and Cysteine

A number of excellent colorimetric and amperometric procedures have been described in detail in our previous monograph (Block and Bolling, 1951, p. 189 *et seq.*); consequently, only a brief mention of some of the newer modifications of these procedures will be described.

a. Winterstein-Folin Phospho-18-Tungstic Acid Procedure. Kolb (1952a, 1952b) carries out the reaction at pH 5.2 in acetate buffer and reduces the cystine to cysteine with zinc dust under nitrogen. He claims a ± 2% precision but points out that the significance of the quantities of cysteine found in protein hydrolyzates is meaningless due to its formation from cystine during hydrolysis by the decomposition of tryptophan.

b. Other Colorimetric Procedures for Cystine and Cysteine. Yoritaka (1953b) has modified the Fleming-Vassal (Block and Bolling, 1951, p. 205 *et seq.*) dimethyl-*p*-phenylenediamine procedure. Pearse (1953) estimated cystine and other -SH compounds in tissues by incubating at 60° C. with tetrazolium salts in 0.1 *M* sodium hydroxide-glycerol buffer at pH 12.8. The concentration of tetrazolium was 25 mg.%. Flesch (1950a) and Scott (1954) employed 1-(-4-chloromercuriphenylazo)-naphthol-2 for sulfhydryl determinations. The color is read with a 520 mμ filter. Dische (1951) used the following simple procedure for cysteine: To 1-10 mg. of cysteine in 1 ml., add 0.65 ml. of H_2O, cool in an ice bath and add 4.5 ml. of a glucose-H_2SO_4 solution (6 parts of conc. H_2SO_4 + 1 part of 10 mg.% aqueous glucose). Allow to stand 3 minutes, add 0.10 ml. of 150 mg.% aqueous tryptophan, warm in water bath to room temperature for 10 minutes and then allow to stand at room temperature for 4 hours. Read using filter 505 mμ. Cystine is determined the same way except that it is first reduced with 20% Na_2SO_3 for 30 minutes (*cf.*, Block and Bolling, 1951, p. 196).

Hird (1954) has modified the brucine method (*cf.*, Block and Bolling, 1951, p. 211) as follows:

Reagents

Glycine sulfate. 5 g. glycine in 100 ml. of 12.5% H_2SO_4.
Background cysteine. 0.2 mg. cysteine per ml. Prepare fresh.
Brucine. 1% in 5% aqueous H_2SO_4.
Potassium persulfate. 0.5% $K_2S_2O_8$.
Trichloroacetic acid. 50% $^w/v$.

Procedure: To 2 ml. of glycine•H_2SO_4, add 1 ml. of background cysteine, 2 ml. of brucine, 2 ml. of trichloroacetic acid filtrate containing 0.0-0.4 mg. of cysteine or cystine and 1 ml. of $K_2S_2O_8$. Develop color at 30 ± 0.1° C. for 45 minutes and cool in ice 2 minutes. Read at 660μ.

c. Amperometric Methods for Sulfhydryl Compounds. (*Cf.*, Block and Bolling, 1951, p. 211.) These procedures are generally not used for the determination of cystine in protein hydrolyzates but rather for free and bound sulfhydryl compounds in tissue fluids and extracts. Some of the pertinent papers are by Carter (1954), Cecil (1950, 1955), Hata (1950), Kolthoff (1950, 1954), Sticks (1953, 1954) and Zweig (1953). Goldzieher (1953) and Larsen (1949) have determined the true -SH groups present in tissues by amperometric titration with silver nitrate from the difference in titer before and after tying up the -SH groups with 0.001 N p-chloromercuribenzoate.

d. Precipitation of Cysteine. Strickland (1954) has reported a modification of the Hopkins cuprous mercaptide method for cysteine and the Beach procedure for methionine (*cf.*, Block and Bolling, 1951, p. 203 *et seq.*), while Ghiglione (1954) precipitated cysteine as the cadmium salt with $CdSO_4$-NaOH at pH 7-8. These procedures are valuable for the isolation of cysteine, homocysteine, glutathione, etc.

e. Manometric Methods. The fact that cysteine, cystine and glutathione will liberate nitrogen from sodium azide-iodide solutions has been used by Holter (1949) and Ishii (1952-1953). The reaction is carried out at pH 7.0 in the Warburg (*cf.*, Block, Durrum and Zweig, 1955, p. 92).

Comments: The observation of Hanes (1950; *cf.*, Smith, E. L., 1955a) that the addition of approximately 2 moles of 0.05 M N-ethylmaleimide at pH 5-8 will react quantitatively with -SH groups has not, to our knowledge, been used as an aid in colorimetric and amperometric methods for cystine, cysteine, etc. This reagent has been used in paper chromatographic techniques (*cf.*, Block, Durrum and Zweig, 1955, p. 95).

2. Methionine

The methods for methionine determination which were described previously (Block and Bolling, 1951, p. 214 *et seq.*) have been modified by the following investigators: the Baernstein method by Schormüller (1951), the Lavine procedure by Bakay (1950) and the McCarthy-Sullivan method by Rudra (1951) and del Amo (1952). Dische (1951) has adapted his cystine method for methionine by using 40 mg.% of glucose in place of 10 mg.% (see Section 1, above); while Müting (1949c) described the following procedure: methionine in a protein hydrolyzate is oxidized with hydrogen peroxide and ammonium molybdate to methionine sulfone (*cf.*, Block, Durrum and Zweig, 1955, p. 94). The methionine sulfone is then reacted with potassium iodide and the liberated iodine is titrated as usual.

Lavine (1953; *cf.*, Floyd, 1954; Weiss, 1955) has isolated methionine as the methylsulfonium salt as follows:

a) Hydrolyze protein with HCl and decolorize with carbon.

b) Precipitate the bases with phospho-24-tungstic acid (*P.W.*). (*Cf.*, Block and Bolling, 1951, pp. 30-33.)

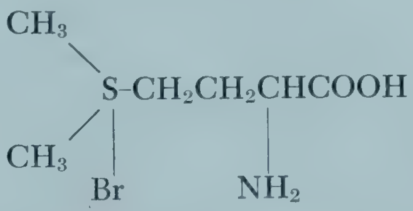
c) Remove excess *P.W.* with tetraethylammonium chloride.

d) Convert methionine to the methylsulfonium derivative with methanol and 18 *N* sulfuric acid.

e) Precipitate methylsulfonium sulfate with *P.W.*

f) Dissolve precipitate in 90% acetone and remove *P.W.* with tetraethylammonium bromide.

g) Neutralize the filtrate with ammonium hydroxide and after decolorizing, concentrate, and isolate methionine methylsulfonium bromide from the methanol solution by precipitation with ether. The formula for this methionine derivative is:



$$\mathrm{(CH_3)_2S(Br){-}CH_2CH_2CH(NH_2)COOH}$$

Noll (1953) claims that methionine can be precipitated at pH 3-4.5 with $CuCl_2$, while Tsuchiya (1949, 1951) oxidized a mixture of methionine and leucine with H_2O_2 to give methionine sulfone. The soluble methionine sulfone was then separated by fractional crystallization and reconverted to methionine by reduction with ammonium sulfite at 100° C. in 2 hours.

3. Some Compounds Related to Cystine and Methionine

a. Glutathione. Grunert (1950) has described a nitroprusside test for this peptide.

b. Lanthionine. This amino acid can be cleaved (Sullivan, 1952) by adding to 5 ml. of lanthionine solution (0.1-0.5 mg. in 0.1 *N* HCl) 1 ml. of 5% aqueous NaCN followed by 1 ml. of cyanogen bromide (decolorize 10 ml. of bromine water at room temperature with 5% NaCN added dropwise). Boil over free flame for 30 seconds and cool at room temperature for 3 minutes. Repeat boiling and cooling twice more. This solution is then diluted to 6 ml. and the following reagents added: 1 ml. of 5% NaCN, 2 ml. of freshly prepared aqueous 1% 1,2-naphthoquinone-4-sodium sulfonate (shake 10 sec.), 5 ml. of 10% Na_2SO_3 in 0.5 *N* NaOH and shake. Allow to stand 30 minutes at 20-25° C. and finally add 1 ml. of freshly prepared 2% $Na_2S_2O_4$ in 0.5 *N* NaOH. Read using filter 540 mμ. Cystine, cysteine, and methionine do not interfere.

c. Cystathionine. This compound has been found in the liver by paper chromatography by Hess (1952a); in horses' hoofs by Shimomura (1950) and in *Neurospora* by Fling (1951).

d. α-Amino-β-(2-amino-2-carboxyethylmercaptol)-butyric acid. Alderton (1953) has reported this compound to be present in subtilin.

SERINE AND THREONINE

Modifications of the periodate methods (*cf.*, Block and Bolling, 1951, p. 264 *et seq.*) for serine and threonine based on the color formation of formaldehyde with chromotropic acid and of acetaldehyde with *p*-hydroxydiphenyl have been described by Bricker (1950), Frisell (1954) and Neidig (1952; *cf.*, Tannenbaum, 1951). Botvinnik (1948) has reported that β-hydroxy-α-amino acids after heating with benzoic acid anhydride at 125-130° C. for 5 minutes and dissolving the reaction mixture in 60% ethanol will decolorize a 0.5% solution of $KMnO_4$, while Fearon (1954) reported that treating serine or threonine (1 mg. per ml.) with 2 ml. of 0.02% aqueous 1,2-dinitrobenzene 20% NaOH 3-6 drops of 1% NaOCl gives a violet color in 10 minutes at room temperature. Phillips (1954) found that hydrolyses of threonine with 6 *N* HCl at 120° C. gave α-amino-*n*-butyric acid, ammonia, carbon dioxide, glycine and an unknown material (R_f 0.90 in phenol). The odor of the hydrolyzate was "pleasant, curry-like." E. Roberts (1954) reported the presence of the orthophosphate diester of L-serine in turtle tissue.

LEUCINE, ISOLEUCINE AND VALINE

1. Leucine

This method was developed by Müting (1949b) and involves boiling the neutralized hydrolyzate with an excess of $CuCO_3$ for 15 minutes, and filtering the hot solution. The excess $CuCO_3$ is then washed with hot water and the filtrate and washings are cooled in ice for 20 minutes after which the precipitate is removed. To the filtrate, containing 5-25 mg. of leucine, is added 5 ml. of 20% copper acetate and 3 ml. of 3% acetic acid. The mixture is boiled for 15 minutes and 5 ml. of absolute ethanol is then added and the solution allowed to cool 20 minutes in ice and filtered. The leucine copper precipitate is dissolved in dilute H_2SO_4 and titrated with *N*/50 sodium thiosulfate. Calculate as 1 ml. of N/50 thiosulfate = 5.24 mg. of leucine.

2. Isoleucine

Löhr (1950) developed the following procedure: oxidize the protein hydrolyzate with ninhydrin at pH 1-2 using 25.4 mg. of ninhydrin per mg. of nitrogen plus 50% excess. Steam distill the aldehydes into 5 ml. of 0.3% 2,4-dinitrophenylhydrazine in 2 *N* HCl (ice cold). Neutralize the distillate with $NaHCO_3$ and extract the hydrazones with CCl_4. Dry the extract and chromatograph on Al_2O_3 (*cf.*, Block and Bolling, 1951, pp. 323, 402) using 5% $^v/v$ methylacetate in petroleum ether as the developing agent. Determine the quantity of isoleucine from the yield of isoamyl hydrazone.

Comments: The separation of methionine from leucine by Tsuchiya (1949, 1951) has been described on page 41. Shildneck (1949) used the following procedure for deaminating amino acids (*cf.*, Block and Bolling, 1951, pp. 296-298): A 15% excess of $NaNO_2$ in 0.5 *N* H_2SO_4 is added to the amino acid solution at 95° C. over a period of 30 minutes. Heating is continued until gas evolution ceases. The hydroxy acids are extracted with ether and dried over $MgCO_3$.

Leucine, isoleucine, valine and other monoamino-monocarboxylic acids can be separated by fractional distillation of their esters and acetylated esters (*cf.*, Block and Bolling, 1951; Mellon, 1953).

THE DICARBOXYLIC AMINO ACIDS

1. Enzymatic Methods of Determination of Glutamic and Aspartic Acids

Meister (1951) observed that suspensions of *Clostridium welchii*

SR12 decarboxylate glutamic and aspartic acids and that the decarboxylation of glutamic acid only is inhibited by semicarbazide. These facts permitted the development of a simple manometric procedure for the determination of the dicarboxylic amino acids.

Krebs (1950) described in detail an enzymatic method for glutamic and aspartic acids based on the following reactions:

$$a)\ \text{L-aspartic acid} + \alpha\text{-ketoglutaric acid} \xrightleftharpoons[\text{transaminase}]{\text{aspartic-glutamic}} \text{L-glutamic acid} + \text{oxaloacetic acid}$$

$$b)\ \text{Oxaloacetic acid} \xrightleftharpoons[\text{decarboxylase}]{\text{oxaloacetic}} CH_3COCOOH + CO_2\uparrow$$

$$c)\ \text{L-glutamic acid} \xrightarrow[\text{decarboxylase}]{\text{glutamic}} \gamma\text{-aminobutyric acid} + CO_2\uparrow$$

Therefore, 2 moles of CO_2 are obtained from 1 mole of L-aspartic acid (*cf.*, Okunuki, 1952). If the enzymes in *a*), *b*) and *c*) are supplemented with asparaginase, then asparagine is also determined.

2. Chromatographic Methods

Modifications of the Al_2O_3 and ion exchange procedures (*cf.*, Block and Bolling, 1951, pp. 323-324) have been described by de Hemptinne (1954), Drake (1952), Fromageot (1949b), Kretovich (1948), Perri (1954), Westall (1950), and Wetzel (1953). It is advisable to remove the aromatic amino acids from the hydrolyzate by passage through H_2S-treated charcoal and to remove any cystine adsorbed on Al_2O_3 by washing the column with aqueous H_2S.

3. Engeland-Dakin Method for Aspartic Acid

This is the procedure of Friedberg (1950); *cf.*, Block and Bolling, p. 334 (1951). Treat 0.6-1.4 mg. of aspartic acid in 1 ml. of water with 20% NaOH until alkaline and add dropwise 0.25 ml. each of dimethylsulfate and 33% NaOH with shaking. Keep in ice box overnight. Acidify with 10 *N* H_2SO_4 to pH 2, add 1 g. of dry SiO_2 gel (Marshall, 1949a) and suspend in 15 ml. of 25% v/v *n*-butanol:chloroform. Filter and wash residue with 20 ml. of solvent. Remove solvent in a current of warm air, dissolve the residue in 1 ml. of water at 70° C. and transfer to a centrifuge tube with 2 *x* 0.25 ml. of H_2O. Precipitate fumaric acid as the copper-pyridyl complex and determine the copper colorimetrically (Marshall, 1949b).

Comments: Wiltshire (1953) reports that hydrolysis with 6 *N* HCl for 24 hours causes about 5% of the glutamic acid in proteins to invert to D-glutamic acid and the *Lactobacillus casei* cells contain approximately 15% of their glutamic in the D-form. Dent found that Dakin's preparations of "β-hydroxyglutamic acid" were mixtures of glutamic acid, aspartic acid, glycine, serine, alanine and surprisingly, taurine. Barker (1952) used the isotope dilution method for the determination of glutamic and aspartic acids while Yoshida (1953) employed electrodialysis to separate aspartic acid from the amino acid filtrate after crystallization of glutamic acid hydrochloride. Synge (1951) found that γ-aminobutyric acid accounted for approximately 6% of the nonprotein nitrogen in rye grasses and we have found this amino acid to account for approximately 15% of the nonprotein nitrogen of orange juice (Block, unpublished).

GLYCINE AND ALANINE

Tannenbaum (1951) uses phenylhydrazine plus potassium ferricyanide in place of chromotropic acid for the determination of formaldehyde (from the ninhydrin oxidation of glycine or the periodate oxidation of serine and hydroxylysine; *cf.*, Block and Bolling, 1951 and Christensen, 1951). Abdel-Akher (1951) points out that glycine is converted to oxalic acid by nitrous acid under conditions used in the Van Slyke method, while Frick (1952) reported that on acid hydrolysis (40% HCl in sealed tubes at 105-110° C. for 24 hours) adenine yields glycine.

Krol (1952) has described a procedure for the isolation of dinitrophenylglycine based on its chromatographic separation on Celite 545 and colorimetric determination of the isolated DNP-glycine in aqueous $NaHCO_3$, filter 601 mμ. Shimizu (1954) improved the nitranilic acid method for glycine (Block and Bolling, 1951, p. 346) by first removing histidine picrate from the hydrolyzate by adsorption on carbon. Glycine and other nonbasic amino acid picrates are not adsorbed (see first section of this Chapter).

PROLINE AND HYDROXYPROLINE

The chemical methods for the imino acids described in Block and Bolling (1951) were not entirely satisfactory; therefore, the newer procedures for these substances are given in greater detail below.

1. Hamilton's Method for the Sum of Proline + Hydroxyproline

All the amino acids in a protein hydrolyzate except proline and hydroxyproline are converted into the α-hydroxy acids by treatment with $NaNO_2$ and 6 *N* HCl (Hamilton, 1950a, 1950b, 1950c; Heyns, 1952a; Block and Bolling, 1951, p. 290; Shildneck, 1949). The excess HONO is destroyed with ammonium sulfonate and the hydroxy acids are extracted with ether. The ether layer is discarded and the aqueous layer, containing proline and hydroxyproline, is oxidized with ninhydrin at pH 1. The liberated CO_2 is collected in $Ba(OH)_2$ and titrated (*cf.*, Block and Bolling, 1951, p. 62) or the CO_2 is determined manometrically.

2. Fearon's Method for the Sum of Proline + Hydroxyproline

To a neutral solution of amino acids, add 1 drop of 25% aqueous aldol and adjust to pH 9.3 by shaking with a slight excess of powdered borax. Then add 2 drops of 1% aqueous sodium nitroprusside. Proline, hydroxyproline and pyrrolidines give a dark blue color (Fearon, 1951). Sulfhydryl compounds give the test and consequently must be oxidized with iodine, etc.

3. Colorimetric Estimation of Proline and Hydroxyproline With Naphthoquinone-4-Sulfonate

This procedure was developed by Troll (1953a, 1953b).

Reagents

1,2-Naphthoquinone-4-sulfonate (*NQS*) twice recrystallized (*cf.*, Block and Bolling, 1951, p. 198). Prepare a 0.02 *M* aqueous solution just before use.

Ascorbic acid. 0.1 *M* ascorbic acid in 0.001 M HCl. (HCl must be free of Fe.)

Method: Add to 1 ml. of protein hydrolyzate (3-10 mg. of protein) in a 50-ml. flask, 1 ml. of 6 *M* $NaNO_2$ and 0.3 ml. of glacial acetic acid. Allow to stand one hour with occasional shaking. Then add 10 ml. of conc. HCl and concentrate by boiling to 2 ml. Repeat addition of HCl and boiling *twice*. Add 2 *N* NaOH until solution is red to phenolphthalein and then add 0.1 *N* HCl to just colorless. Then add 1 ml. of *M* $NaHCO_3$, 2 ml. of *NQS* and dilute to 10 ml. with water. Stand 10 minutes and add 1 ml. of *M* acetate buffer, pH 5, and 1 ml. of ascorbic acid solution. Dilute to 25 ml. and read using filter 480 mμ against a reagent blank of all ingredients except *NQS*.

Comments: Proline and hydroxyproline can be separated on Dowex-50 (200-400 mesh) using citrate buffer at pH 3.30 (Rogers,

1954) or on paper (Troll, 1953b). Creatine, which interferes, must be removed chromatographically. A solution of *NQS* in 0.1 *M* $NaHCO_3$ can be used to detect proline and hydroxyproline, after destroying the α-amino acids with HONO, on paper chromatograms (Troll, 1953b).

4. Determination of Proline and Pipecolic Acid

This is the method of Schweet (1954).

Reagents

Ninhydrin. 372 mg. in 20 ml. of glacial acetic acid. Prepare just before use.
Glacial acetic acid. Preserved from moisture.
Concentrated HCl:acetic acid = 1:99 v/v.
HCl, 8.6 *N*. 10 ml. conc. HCl + 4 ml. H_2O.
$NaNO_2$, 3 *M*. Dissolve 20.7 g. in 100 ml. H_2O. Prepare fresh.

Method: To 1 ml. of HCl hydrolyzate add 1 ml. of 0.3 *M* $NaNO_2$, 0.7 ml. of 8.6 *N* HCl and heat in an oil bath at 122° C. for 2.5 minutes with shaking. Cool in ice to room temperature. Remove anions with Amberlite IR-4B to pH 4 or above. Adjust proline concentration to approximately 25-40 γ per ml. with glacial acetic acid. Using 0.1 or 0.2 ml. aliquots, add water to bring *final* content to 0.05 ml. Then add glacial acetic acid to 3.85 ml. and 0.3 ml. of ninhydrin. Cover tubes, heat at 121-122° C. for 5 minutes, and cool in ice bath to room temperature. Read color at 530 mμ within 15 minutes against reagent blank.

Comment: Ornithine interferes. The above method is for proline. The method for pipecolic acid is the same except that the HCl is omitted, the heating is carried out for 8 minutes and the color is read at 560 mμ.

5. Determination of Proline

This method was developed by Chinard (1952) and modified by Troll (1955).

Reagents

Proline standards, 1 to 5 x 10^{-5} *M*.
Ninhydrin. 125 mg. of ninhydrin are dissolved in 3 ml. of glacial acetic acid and 2 ml. of 6 *M* H_3PO_4 by heating to 70° C. The reagent is stable for 24 hours.
Permutit, "according to Folin."

Method: Ten mg. of protein are hydrolyzed with 1 ml. of 6 *N* HCl in a sealed tube at 110° C. for 24 hours. The hydrolyzate is diluted to 250 ml. with water and shaken with sufficient Permutit to remove the basic amino acids (*cf.*, Block and Bolling, 1951, p. 36). To 5 ml. of the filtrate, add 5 ml. of acetic acid and 5 ml. of ninhydrin reagent. Heat in a water bath for one hour in covered test tubes. Cool to room temperature and extract the color with 5 ml. of benzene by vigorous shaking for 5 minutes. Separate the benzene layer and read at 515 mμ.

Comment: Cysteine interferes with this procedure.

6. Determination of Hydroxyproline*

This is the method of McFarlane (1939) described in Block and Bolling, 1951, p. 367-369, as modified by Neuman (1950a).

Reagents

Hydroxyproline standards. 5, 10 and 15 γ per ml.
Copper sulfate. 0.1 *M* $CuSO_4$.
Sodium hydroxide. 2.5 *N* NaOH.
Hydrogen peroxide. 6% H_2O_2.
Sulfuric acid. 3 *N* H_2SO_4.
p-Dimethylaminobenzaldehyde, 5% in *n*-propanol. The *p*-$(CH_3)_2$-NC_6H_4CHO was recrystallized according to Adams (1933) from 1:6 $^v/v$ aqueous HCl by fractionation with 15% NaOH.

Method: To standards and unknowns (1 ml. each) add 1 ml. of $CuSO_4$, 1 ml. of NaOH, and 1 ml. of H_2O_2. Mix and shake from time to time during 5 minutes. Warm in a water bath for 5 minutes at 80° C. with frequent vigorous shaking to destroy the H_2O_2. Cool in an ice bath and add 4 ml. of H_2SO_4, shake and add 2 ml. of *p*-$(CH_3)_2NC_6H_4CHO$. Mix and warm at 70° C. in a water bath for 16 minutes, cool in tap water and read using a 540 mμ filter against a reagent blank (*cf.*, Consden, 1953).

7. Martin's (1953) Modification of Neuman's Method for Hydroxyproline

Use 2 ml. of hydroxyproline solution. Add 0.1 ml. of *M*/20 $FeSO_4$ in 0.5% $^v/v$ H_2SO_4 to remove excess H_2O_2. Shake for 6 minutes. Mix $CuSO_4$ and NaOH *just* before use. Add H_2SO_4 and *p*-$(CH_3)_2NC_6H_4$-

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* Recommended method.

CHO simultaneously. Increase concentration of H_2SO_4 from 3 *N* to 4 *N*. Decrease concentration of $p\text{-}(CH_3)_2NC_6H_4CHO$ from 5% to 4%.

Comments: Although the Neuman-Martin *p*-dimethylaminobenzaldehyde method for hydroxyproline appears to be the best available, Wiss (1949) and Müting (1952a) have described other modifications. Mason (1955) has reported that catechol or 4-methyl catechol will give color with the *N*-terminal proline of proteins, while Friedberg (1954) has been able to separate hydroxyproline and allo-hydroxyproline by paper chromatography as follows: Place 25 mg. of amino acid on paper and spray the spot with saturated copper acetate in tetrahydroquinone containing 5% H_2O and 1% acetic acid. Develop with phenol:water:acetic acid = 80:20:2 $^v/v$. Dry the paper and spray with ninhydrin. Hydroxyproline, $R_f = 0.59$; allo-hydroxyproline, $R_f = 0.75$.

Radhakrishnan (1954) describes the isolation of hydroxyproline.

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CHAPTER IV

Microbiological Methods for the Determination of Amino Acids

By **Sidney Saperstein, Ph.D.**

INTRODUCTION

ALTHOUGH biological methods for the determination of the amino acids were first introduced by Osborne and Mendel (1914), and are still in use for the assay of amino acids in proteins and foods, the results so obtained are seldom considered to be sufficiently reliable because of the limited number of test subjects employed. Unless a vast number of animals is used in such experiments, it becomes exceedingly difficult, if not impossible, to obtain a numerical expression for the amino acid composition of the protein. For example, the large discrepancy between the cystine value of casein as determined by the rat growth method (Sherman and Woods, 1925) and that found by the highly accurate chemical procedures, suggested to Jackson and Block (1931, 1932) that methionine might assume all or part of the nutritive functions of cystine. The results of these and other investigations eventually led to the discovery of the essential nature of methionine.

The development and presentation of various protocols employing microorganisms for the qualitative and quantitative determination of amino acids and other essential metabolites, reflects the rapid progress made in recent years in the field of nutrition and physiology. Since the discovery of "bios" by Wildiers (1901), the endeavors of many workers on related problems reached fruition when certain similarities between the nutritional requirements of plants and animals was established (Lwoff, 1938; Rose, 1938; Knight, 1945). It is primarily to the success of Snell and his coworkers (1937, 1939) that the groundwork for present day microbiological assay techniques is to be credited. The investigation of Wood (1940) first suggested the use of microorganisms for the determination of amino acids as a sequel to the progress made with this technique in the determination of the B complex vitamins. These assay methods, in many instances, are superior to most other methods except the elaborate physiochemical procedures such as isotope dilution and the better systems of chromatography. The microbiological method presents a convenient and accurate means for the estimation of eighteen of the known amino acids.

GENERAL ASPECTS OF THE MICROBIOLOGICAL TECHNIQUE

Fundamentally, all microbiological assay methods in current use are so similar in technique, that once a procedure is mastered for any one amino acid, it can be applied with equal facility to any other for which suitable conditions have been found. The value of this, especially for routine analysis, is apparent.

It should be remembered, however, that growth of living cells is subject to many more extraneous influences than are the *in vitro* chemical reactions or physical measurements (*cf.*, Kidder, 1949). Results obtained by the biological methods, therefore, must be evaluated by one competent in the field. The analyst must be alert to the fact that while, in the main, most organisms respond only to the natural form of the amino acids (Lyman, 1943; Stokes, 1944), in several instances the D-enantiomorph and even the keto analogues (Eades, 1950) are equally effective in promoting growth (Camien, 1950b; Sauberlich, 1949). Again, it is not uncommon to observe stimulatory or inhibitory responses elicited by the D-isomer (Prescott, 1949; Rydon, 1948).

The presence or absence of certain vitamins (Holden, 1951; Horn, 1953; Lascelles, 1954; Lyman, 1948; Stokes, 1945), lipoid substances (Williams, V. R., 1945), oxygen (Bohonos, 1942), carbon dioxide (Lascelles, 1954) or certain antagonistic amino acids (Gladstone, 1939) may greatly influence the growth of the test organism, even to the extent of determining the "essentiality" of an amino acid for the organism. Sparing of an amino acid by the addition of other amino acids or compounds (McClure, 1954) is not uncommon. The rate and amount of growth of a microorganism is also influenced by temperature. It is therefore not surprising to find, in several instances, that the nutritional requirements of the organism are affected by temperature. An example of this is seen in the work of Borek (1951) who demonstrated that *Lactobacillus arabinosus* required phenylalanine when cultured at 37° C.; whereas, at 35° C., phenylalanine was neither required nor stimulatory for growth of the organism.

Since the lactic acid bacteria are the most commonly employed

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organisms for amino acid assays, it would be well to remember their requirement for low oxidation-reduction potentials. These microaerophilic bacteria will generally grow without difficulty in the volumes of medium routinely employed in assays (5-10 ml.); however, if very small volumes are used (less than 5 ml.), inhibitory effects may be encountered (Lowry, 1944; *cf.*, Ågren, 1949b). The large amount of acid produced by these organisms necessitates some control over the hydrogen ion concentration during the course of the assay. The choice of suitable buffers is important and care must be exercised that the buffer does not chelate essential minerals as is the case with excess citrate ions (MacLeod, 1947).

Closely related amino acids may affect the results of the assay; *e.g.*, glutamine in the glutamic acid analysis (Fildes, 1939; Lyman, 1945), leucine, valine, and methionine in the analysis of isoleucine (Brickson, 1948), and arginine in the assay for proline (Sirny, 1951).

Simple peptides have also been shown to be responsible for stimulatory or inhibitory effects on amino acid assays (Woolley, 1941; Dunn, 1950; Krehl, 1948; Peters, 1954; Prescott, 1953). The presence of such substances can easily lead to invalid assays. While most amino acid assays are carried out on completely hydrolyzed proteins, natural substances, such as body fluids and cereal grains, may not lend themselves to complete hydrolysis, thereby negating an essential criterion for microbiological assays. Klungsøyr (1951) studied this problem with regard to assays employing the lactobacilli. He found that several lactobacilli were stimulated by lower peptides of phenylalanine and tyrosine during short incubation periods. Horn (1953) demonstrated the stimulatory nature of humin (or substances carried along with humin) in the assay of cereals and legumes for several amino acids.

Although some of the biological methods are suitable for application to crude protein-containing foods, *e.g.*, leucine, isoleucine and valine (Lyman, 1943; Schweigert, 1945), assays for other amino acids cannot be applied to natural products because of toxicities encountered at the higher dosage levels (Stokes, 1945; Macrae, 1947). In fact, in the light of our present knowledge, it would seem advisable to re-examine some of the earlier described assays for crude proteins (Horn, 1955).

It should be recalled that with few exceptions (Regnery, 1944; Rockland, 1946; Hodson, 1947) microbiological procedures for the amino acids requires partial or complete hydrolysis of the protein and are therefore subject to the same losses applicable to chemical methods. This point is often forgotten when the relative merits of microbiological* and chemical procedures are discussed.

Although the use of various fungi for assay purposes is waning, the application of protozoal methods is being given greater consideration in recent years. The chief advantage of the protozoal technique is that these lower animals can ingest intact protein, eliminating the need for hydrolysis of the test substance prior to the assay. Rockland (1946) reported the use of *Tetrahymena geleii* (H) for the estimation of tryptophan in undigested casein (see also Dunn, 1947b; Williams, H. H., 1951; Pilcher, 1954; Viswanatha, 1955). Hutner (1955) has indicated that it may soon be possible to assay for most of the amino acids using the protozoal method. This potentially useful method, however, requires further development.

NUTRITIONAL REQUIREMENTS OF MICROORGANISMS

An organism if it is to grow and reproduce must be supplied with those essential substances that can be transformed into new protoplasm, as well as those substances which can serve as a source of energy for this transformation.

For this synthesis to occur, therefore, microorganisms require sources of carbon, nitrogen, inorganic salts and energy. Some organisms can synthesize all of their protoplasm from carbon dioxide and ammonia plus a source of energy; however, many forms of life do not possess such great synthetic capabilities. Lacking the ability to make use of simple substances, a microorganism must be supplied with the essential nutrilites if it is to grow. Studies on the nutrition of all organisms involve a determination not only of the essentials necessary for growth, but also the optimal concentrations and combinations of various substances which will permit initiation and continuation of growth. A fundamental problem in the nutrition of an organism is the construction of a medium of known composition capable of eliciting these desired optima. The many studies in this field led to the elucidation of what has been termed synthetic disabilities in the heterotrophic microorganisms. Unable to synthesize a particular nutrilite, an organism is said to be nutritionally "exacting" towards this essential metabolite. Whereas it has not been difficult to find carbon, nitrogen, and mineral components which could be utilized by many bacteria, for others, extracts of plant and animal tissues were found to be necessary for various physiological processes. The culmination of these studies on the nutritional requirements of these "exacting" organisms has enabled the microbiologist and biochemist to select microorganisms for use in the assay of amino acids to be covered here.

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* The determination of an amino acid based upon its decomposition by specific enzyme systems is also included among the microbiological techniques. However, these methods are not in too common use, and will not be covered here.

It is now possible, through the application of the microbiological assay, employing several species of the lactic acid bacteria, to analyze quantitatively for eighteen of the known amino acids. As our knowledge of the metabolic requirements of these organisms has grown, improvements on the earlier methods have been achieved, and no doubt will continue.

From the foregoing, one can readily see that the basic principle underlying the microbiological assay is a straightforward extension of the related nutritional studies. A basal medium is prepared which contains all the essential metabolites for the test organism, except the one to be determined. The addition of increasing amounts of the substance under test to the basal medium, results in a growth response which is a direct function of the concentration of the test substance added. The physiological response of the organism to the test substance (cell number, cell mass, acid production, cell nitrogen, *etc.*) can be conveniently measured after inoculation and incubation. Simultaneously, a parallel run is made to which graded amounts of the pure material are added to the basal medium. The physiological response of the organism at the conclusion of the incubation period is then plotted on a suitable graph paper. From the two curves obtained, response curve and standard curve, respectively, the amount of active material can be determined by interpolation, or by other means which will be discussed later (*cf.*, Maccaro, 1954).

MATERIALS AND METHODS

1. Glassware

The use of borosilicate glassware (Corning Glass Co. or Kimble Glass Co.) is strongly recommended for both the assay tubes as well as for the stock solutions. All glassware should be thoroughly cleaned by boiling in suitable detergent (Borden's "Super SS"). Stainless steel or aluminum vessels can be used for boiling the glassware. The use of detergents seems to be superior to other methods of cleaning, such as chromic-sulfuric acid or nitric acid-potassium nitrate mixtures. The use of chromate solutions introduces the hazard of incomplete removal of this toxic ion. The acid solutions also seem to be inferior for removing greasy deposits.

Pipettes are conveniently cleaned by placing them vertically in a stainless steel carrier which is then placed in a tall stainless steel cylinder having sufficient water capacity to cover the pipettes. The detergent solution is brought to a boil and held there for at least fifteen minutes. The pipettes are removed with the carrier and placed in a suitable rinsing apparatus. Thorough rinsing is essential. Tap water is generally used, followed by a final rinse with distilled or deionized water.

2. Sterilization

For aseptic operations, pipettes are sterilized individually in glass tubes cleaned as above. The tubes are closed at one end by crimping some glass wool over the end of the tube. The other end of the tube is closed with gauze-covered cotton plugs. The pipettes are autoclaved at 121° C. (15 lbs./sq. in.) for 30 minutes. Following sterilization, the pipettes are allowed to dry at room temperature. Autoclaves designed for drying material after sterilization are particularly useful in this step.

The media employed in the various assays are sterilized by autoclaving at 115° C. (10 lbs./sq. in.) for 10 minutes. Maintenance media are sterilized at 121° C. for 15 minutes. Stock solutions are best preserved by the addition of 0.5% of the following volatile preservative (Hutner, 1953): chlorobenzene:1,2-dichloroethane:1-chlorobutane = 1:1:2 $^{v}/v$. The preservative is distilled off during autoclaving and therefore presents no difficulty in handling.

3. Stock Solutions

The following stock solutions are prepared with glass distilled water containing potassium permanganate in the distilling flask. Except for the inorganic salt solutions, storage in a refrigerator at 4-8° C. is necessary.

Acid-Hydrolyzed Casein: Suspend 200 g. of Labco "Vitamin-Free" casein in 800 ml. of 10% sulfuric acid. Heat this suspension for six hours in the autoclave at 121° C. (15 lbs./sq. in.). Add barium hydroxide to the hydrolyzate to adjust the pH to 2.5-2.8. To this hydrolyzate add 10 g. of activated carbon (Darco G-60) and either shake the resulting mixture for 30 minutes or keep at a very slow boil for this length of time. Remove the activated carbon and barium sulfate precipitate by filtration with the aid of Celite or other suitable filter-aid. Wash the filter cake *in situ* with 50-100 ml. of distilled water and adjust the pH of the filtrate to 6.8 with potassium hydroxide. Add 5 g. of Darco G-60 to the filtrate and again shake or boil the mixture as before. Remove the activated carbon by filtration and adjust the volume of the filtrate to 1 liter. The final hydrolyzate is a 20% solution.

Biotin: Dilute an ampoule containing 25 γ of the free acid to 250 ml. with distilled water. The solution contains 0.1 γ of biotin per ml. An alternate procedure is to prepare the stock solution from the crystalline vitamin.

L-*Cystine:* Dissolve 4 g. of L-cystine in 500 ml. of water containing 5 ml. of concentrated hydrochloric acid. Heat the mixture until the cystine is dissolved and adjust the volume to 1 liter. The solution contains 4 mg. of L-cystine per ml.

p-Aminobenzoic Acid: Dissolve 100 mg. of *p*-aminobenzoic acid in 100 ml. of water containing 1 ml. of glacial acetic acid. For routine use dilute this solution to give a solution containing 100 γ of the vitamin per ml. (1:10 dilution).

Riboflavin: Add 1 ml. of glacial acetic acid to 25 mg. of riboflavin and dilute to 1 liter with water. The final solution contains 25 γ of riboflavin per ml. Protect the solution from light during storage; low actinic glassware, or covering the bottle with black paper is adequate precaution.

Thiamine, Nicotinic Acid, Calcium d-Pantothenate and Pyridoxine: Prepare separate stock solutions of each of these vitamins by dissolving 0.1 g. of the material in 100 ml. of water. Dilute aliquots of each of the solutions 1:10 for use in the assays. Protect the solution of pyridoxine from the light.

Pyridoxal: Prepare a stock solution containing 1 mg. per ml. by dissolving 0.1 g. of pyridoxal in 100 ml. of water. Protect the solution from the light.

Folic Acid: Dissolve 1 mg. of the vitamin in 250 of 50% ethanol. Add dilute potassium hydroxide to bring the pH to 7.0 and adjust the volume to 500 ml. with 50% ethanol. Store the solution in the dark.

Folinic Acid: Dissolve 1 mg. of calcium leucovorin in 100 ml. of water. Store the solution in the frozen state. The pH should be maintained above 7 by the addition of sodium hydroxide if necessary.

DL-*Tryptophan:* This solution is used in the preparation of media other than that for the tryptophan assay. Dissolve 4 g. of the DL-isomer in 200 ml. of water with the aid of a few drops of concentrated hydrochloric acid and heat. Dilute to a final volume of 1 liter (4 mg./ml.).

Adenine, Guanine and Uracil: Prepare a single stock solution of these substances containing 1 mg. per ml. by heating the material in water with a few drops of concentrated hydrochloric acid. Make up to volume after the material is dissolved (100 mg. of each in 100 ml.).

Xanthine: To prepare a solution containing 1 mg. per ml., dissolve 100 mg. of xanthine in 100 ml. of water with the aid of a few drops of ammonia.

Salt Solution A: This solution consists of 25 g. of KH_2PO_4 and 25 g. of K_2HPO_4 in 250 ml. of water.

Salt Solution B: This salt solution contains 10 g. of $MgSO_4 \cdot 7H_2O$, 0.5 g. of $MnSO_4 \cdot 4H_2O$ and 0.1 g. of $FeCl_3$ in 250 ml. of water. Addition of a few drops of hydrochloric acid helps prevent the formation of a precipitate in the solution.

Salt Solution C: This consists of 10 g. of $MgSO_4 \cdot 7H_2O$, 0.5 g. of NaCl, 0.5 g. of $FeSO_4 \cdot 7H_2O$, 0.5 g. of $MnSO_4 \cdot 4H_2O$ plus distilled water to 250 ml.

Bromothymol Blue: Dissolve 0.1 g. of the indicator in 16 ml. of 0.01 *N* sodium hydroxide. Adjust the volume of the solution to 250 ml. with distilled water. Five drops of the solution are added to each 10 ml. portion of culture medium to be titrated. The end point (pH 6.9-7.0) is indicated by a blue-green color.

4. Procedure

The following details apply for all assays covered here. The concentrations of the component parts of the various media, as well as the preparation of the inoculum, will be discussed separately for the individual assays.

The usual procedure is to set up the test tubes (16 × 150 mm., Pyrex) in wire racks. Five milliliters of the basal medium, prepared as a double strength solution, are added to each tube. The material under examination, following suitable dilution, is then added to the assay tubes. Three or four different levels of material under test are employed. For each different level, quadruplicate tubes are set up.

A standard response curve is run parallel with the assay. This is accomplished by adding graded amounts of the pure amino acid to duplicate tubes containing the basal medium as described above. For the standard response curve, at least six levels of the amino acid are employed. The amount of the pure amino acid added per tube will vary from zero to the amount necessary to give maximum response by the assay organism. In all the assays, it is well to point out to the novice, that it is the amount of amino acid that we wish to determine and not that of the hydrochloride. When standards are prepared from hydrochlorides, it is necessary to apply the proper correction factor for converting this value to the amount of pure amino acid.

When all the additions have been made, sufficient distilled water is added to each of the tubes to bring the final volume to 10 ml. As an example, an assay for tryptophan in casein would require a total of 28 tubes; 12 tubes for the standard response curve (six graded increments in duplicate) and 16 tubes for the actual assay (four levels in quadruplicate). Many workers consider it good practice to run recovery checks on the assay. This is accomplished by setting up duplicate tubes at two levels with the test substance, as described above, and then adding to each of these tubes a known amount of the pure amino acid. At the conclusion of the assay, one can readily determine the per cent recovery of the known amino acid added.

After the tubes have been set up and diluted to final volume, they are plugged with cotton or covered with aluminum caps and sterilized. The tubes are allowed to cool slowly in the autoclave to prevent unequal losses by distillation of water from the tubes. Upon cooling, the tubes are inoculated with the assay organism and placed in an incubator at a suitable temperature (30° C.) for the required length of time (usually 72 hours).

With the lactobacilli, growth response is measured by titrating the amount of acid produced with 0.1 *N* sodium hydroxide. The response *versus* dose is plotted. From the resultant curve, the amount of amino acid in the test substance can be calculated.

THE ASSAYS

1. α-Alanine

Organism: Leuconostoc citrovorum 8081* *(Pediococcus cerevisiae)* has been suggested for this assay by Sauberlich (1949). The basal medium given in Table II is essentially that suggested by these workers (*cf.*, Steele, 1949).

Basal Medium: The concentrations given in Table II are for a double strength solution. The amino acids are dissolved by heating in distilled water. A few drops of 6 *N* hydrochloric acid may be added to effect rapid solution. After the addition of the other components, the pH is adjusted to 6.8 with potassium hydroxide. In lieu of leucovorin, 0.4 ml. of Lilly's liver concentrate (Reticulogen, 20 U.S.P.) may be used per 500 ml. of double strength medium.

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* Culture obtainable from the American Type Culture Collection, Washington, D. C.

TABLE II

BASAL MEDIUM FOR α-ALANINE ASSAY*

L-Arginine·HCl	242 mg.	Glucose	25 g.
L-Asparagine	400 mg.	Sodium acetate·$3H_2O$	33 g.
DL-Aspartic acid	100 mg.	Ammonium chloride	3 g.
L-Cysteine	50 mg.	Salt solution A	6 ml.
L-Glutamic acid	300 mg.	Salt solution C	2 ml.
Glycine	100 mg.	Adenine sulfate·H_2O	10 mg.
L-Histidine·HCl·H_2O	62 mg.	Guanine·HCl·$2H_2O$	10 mg.
DL-Isoleucine	250 mg.	Uracil	10 mg.
DL-Leucine	250 mg.	Xanthine	10 mg.
L-Lysine·HCl	250 mg.	Thiamine·HCl	500 γ
DL-Methionine	100 mg.	Ca-D-pantothenate	250 γ
DL-Phenylalanine	100 mg.	Riboflavin	500 γ
L-Proline	100 mg.	Nicotinic acid	1000 γ
DL-Serine	50 mg.	*p*-Aminobenzoic acid	100 γ
DL-Threonine	200 mg.	Biotin	1 γ
DL-Tryptophan	40 mg.	Folic acid	10 γ
L-Tyrosine	100 mg.	Leucovorin (Folinic acid SF)	0.5 γ
DL-Valine	250 mg.	Distilled water *q.s.*	500 ml.
		pH 6.8	

* Amount sufficient for 100 tubes.

Preparation of Inoculum: The organism is maintained in stab culture on the following medium: 1.5% agar, 1% glucose and 1% Bacto Yeast Extract (Difco). Transfers are made monthly to new tubes and the cultures are held at 5-8° C. after initiation of growth at 37° C. In preparing the inoculum, a tube of single strength medium, to which 0.1% yeast extract has been added, is inoculated from the stab culture. The inoculum tube is incubated for 24 hours at 37° C. The cells are aseptically centrifuged down and washed *in situ* with 10 ml. of distilled water after decanting the supernatant solution. The cells are again brought down by centrifuging and resuspended in distilled water (an amount equal to the volume of the original medium in the tube). One drop of this suspension is used to inoculate each assay tube.

*Standard α-Alanine Solution:** The organism will respond to α-alanine in the range of 0-25 γ of the amino acid. It is equally responsive to both the D- and L-isomers. A standard solution of the amino acid is made up after drying at 60° C. under 20 mm. of mercury for at least 2 hours. Solution of the amino acid is effected by the addition of a few drops of 6 N hydrochloric acid.

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* All standard solution ranges indicate γ of amino acid per ml.

Preparation of the Hydrolyzate for Assay: An acid hydrolyzate of the protein to be assayed is used (*cf.*, Chapter II). Before use, the hydrolyzate must be neutralized or made slightly alkaline and then exposed to direct sunlight or to ultraviolet radiation. This step is necessary in order to destroy any pyridoxal or pyridoxamine. In the presence of either of these two substances there is a definite growth stimulation of the organism which results in high blanks and a decrease in the sensitivity of the assay. Substances containing lipid material should previously be defatted (*cf.*, Chapter II).

The Assay: Five ml. of the double strength basal medium is placed in the tubes. After suitable dilution, the test material is added to one set and the pure α-alanine is added to the second set (standard curve) as outlined in Section 4 of this chapter (page 57). The volume in each tube is adjusted to 10 ml. and sterilized. The tubes are inoculated when cool and then incubated for 72 hours at 37° C. The amount of acid produced is determined by titrating electrometrically or with an internal indicator (bromothymol blue) using 0.02 N sodium hydroxide.

Calculation: The most convenient and accurate method for determining the amino acid content in the sample from the data is to plot the logarithm of the number of milliliters of alkali (ordinate) against the logarithm of the dose (abscissa). The same is done in establishing the standard curve. This plot is most conveniently constructed by using logarithmic paper. Details and examples of sample calculations are given in the last part of this Chapter (page 68).

2. Arginine, Lysine and Threonine

Organism: Streptococcus faecalis 9790 has been widely used for these amino acid determinations and is recommended for use here. Steele (1949) has recommended the use of *Leuconostoc citrovorum* for the arginine assay. An ultra micro method for arginine has been proposed by Agren (1949b) and by Camien (1954).

Basal Medium: The medium given in Table III is essentially that suggested by Stokes (1945c). The medium is prepared as described in the preceding section. The amino acid to be determined is omitted from the medium.

Preparation of the Inoculum: Streptococcus faecalis is maintained on a medium consisting of 1% glucose, 0.5% Bacto-Tryptone, 0.6% anhydrous sodium acetate, 1.5 ml.% Salt Solution *A*, 0.2 ml.% Salt Solution C, 1.5% agar; the pH adjusted is to 6.8. Transfers are made monthly from cultures held at 5-8° C. The inoculum is prepared by transferring some cells from the stab culture to the inoculum medium. The latter is the same as the maintenance medium except that the agar is omitted. The culture is incubated at 37° C. for 24 hours. The cells are washed as described in the previous assay and then diluted with 100 ml. of sterile distilled water. One drop of this suspension is used to inoculate each assay tube.

TABLE III

BASAL MEDIUM FOR ARGININE, LYSINE AND THREONINE ASSAY*

DL-Alanine	200 mg.	Glucose (anhydrous)	10 g.
L-Arginine•HCl**	200 mg.	Sodium acetate•3H_2O	10 g.
DL-Aspartic acid	200 mg.	Adenine sulfate•H_2O	5 mg.
L-Cystine	200 mg.	Guanine•HCl•2H_2O	5 mg.
Glycine	200 mg.	Uracil	5 mg.
L-Histidine•HCl•H_2O	200 mg.	Xanthine	5 mg.
DL-Isoleucine	200 mg.	Thiamine•HCl	200 γ
DL-Leucine	200 mg.	Ca-*d*-pantothenate	200 γ
DL-Methionine	200 mg.	Riboflavin	200 γ
L-Lysine•HCl**	100 mg.	Nicotinic acid	200 γ
DL-Phenylalanine	200 mg.	Biotin	0.2 γ
L-Proline	200 mg.	Folic acid	2 γ
DL-Serine	200 mg.	Pyridoxamine	400 γ
DL-Threonine**	200 mg.	*p*-Aminobenzoic acid	40 γ
L-Tyrosine	200 mg.	Salt solution *A*	5 ml.
DL-Valine	200 mg.	Salt solution *C*	2 ml.
L-Glutamic acid	100 mg.	Water *q.s.*	500 ml.
DL-Trytophan	400 mg.	pH 6.8	

* Double strength medium.
** Amino acid to be assayed is omitted from the medium.

Standard Solutions of Arginine, Lysine and Threonine: The organism will respond to arginine over the range of 0-60 γ. The effective range for establishing the standard curve for L-*lysine* is 0-200 γ. The range for DL-threonine is 0-120 γ. It is well to remember in establishing the standard curve that some solutions are prepared from racemic mixtures and some may also be prepared from amino acid hydrochlorides. This must be taken into account to avoid mathematical errors, *i.e.*, L-lysine•HCl, L-arginine•HCl and DL-threonine.

The preparation of the hydrolyzate as well as the setting up of the assay and relative calculations are the same as described for the α-alanine assay.

3. Aspartic Acid

Organism: Hac (1945) recommends the use of *Leuconostoc mesenteroides* P-60 for this assay. Other organisms have also been used (Stokes, 1945; Meister, 1950). In the following procedure *L. mesenteroides* P-60 is employed.

Basal Medium: The basal medium of Barton-Wright (1946) seems to be suitable for this assay when *L. mesenteroides* P-60 is employed. The composition of the medium, modified slightly, is given in Table IV.

TABLE IV

Basal Medium for Aspartic Acid Assay*

DL-Alanine	1 g.	Sodium acetate$\cdot$3H$_2$O	20 g.
L-Arginine$\cdot$HCl	250 mg.	Sodium chloride	5 g.
L-Cystine	100 mg.	Ammonium chloride	6 g.
L-Glutamic acid	500 mg.	Adenine sulfate$\cdot$H$_2$O	10 mg.
Glycine	100 mg.	Guanine$\cdot$HCl$\cdot$2H$_2$O	10 mg.
L-Histidine$\cdot$HCl$\cdot$H$_2$O	100 mg.	Uracil	10 mg.
DL-Isoleucine	200 mg.	Xanthine	10 mg.
L-Leucine	100 mg.	Salt solution *A*	5 ml.
DL-Methionine	100 mg.	Salt solution *B*	5 ml.
DL-Phenylalanine	100 mg.	Ca-*d*-pantothenate	1 mg.
L-Proline	100 mg.	Thiamine$\cdot$HCl	1 mg.
DL-Serine	100 mg.	Nicotinic acid	2 mg.
DL-Threonine	500 mg.	Riboflavin	2 mg.
DL-Tryptophan	200 mg.	Pyridoxal$\cdot$HCl**	50 γ
L-Tyrosine	100 mg.	*p*-Aminobenzoic acid	50 γ
DL-Valine	200 mg.	Biotin	5 γ
L-Lysine$\cdot$HCl	100 mg.	Water *q.s.*	500 ml.
Glucose	20 g.	pH 6.8	

* Double strength.

** Pyridoxine$\cdot$HCl (1.6 mg.) may be substituted.

Preparation of the Inoculum: The culture is maintained in a manner similar to that described for *Leuconostoc citrovorum* in the α-alanine assay. The inoculum is prepared similarly except that the culture as well as the assay tubes are incubated at 30° C.

Standard Solution of Aspartic Acid: The organism will respond effectively over the range of 0-50 γ of L-aspartic acid.

The remainder of the procedure is the same as for the assays described previously. It is advisable to use 0.1 *N* sodium hydroxide for the titration.

4. Cystine, Methionine and Tyrosine

Organism: Barton-Wright (1948) (*cf.*, Camien, 1950a; Hutchin, 1950) recommends the use of *Leuconostoc mesenteroides* P-60 for the assay of the L-isomers of these amino acids. For DL-methionine, *Lactobacillus fermenti* 36 may be employed. Shockman (1954) claims increased sensitivity (0-0.22 γ/ml.) with his method. The latter is based on turbidimetric determination of growth with very small volumes of medium (3 ml.). Sterilization of his medium for 3 minutes at 121° C. is claimed to result in no destruction of cystine.

Basal Medium: The basal medium described in Table IV may be used for the determination of L-methionine, L-cystine and L-tyrosine after the following changes; aspartic acid is added to the medium (400 mg./500 ml.) and the amino acid to be assayed is omitted.

Preparation of the Inoculum: The culture of *L. mesenteroides* P-60 is prepared as described for the aspartic acid assay.

Standard Solution of Amino Acids: The range for the standard curve is as follows: L-cystine, 0-35 γ; L-methionine, 0-40 γ; and L-tyrosine, 0-50 γ.

Preparation of the Hydrolyzate: Acid hydrolysis is used for the cystine and methionine assay. For the tyrosine assay, alkaline hydrolysis should be used (*cf.*, Chapter II). However, alkaline hydrolysis results in racemization of the amino acid and the results must be interpreted accordingly. The organism does not respond to the unnatural isomer.

The assay is conducted according to the general procedure outlined above. The incubation time is 72 hours at 30° C. and titration is with 0.1 *N* alkali.

If DL-methionine is to be assayed, the medium described in Table IV may be used with the following modifications: 400 mg./500 ml. of DL-aspartic acid is added and DL-methionine is omitted. In addition to this change, 2 γ of folic acid are added to the medium and the pH is adjusted to 6.4 instead of 6.8.

Comment: Lactobacillus fermenti 9338 is used for the assay of DL-methionine as it responds to both isomers.

Preparation of the Inoculum: L. fermenti is maintained in a manner similar to that described for *Leuconostoc citrovorum*. The inoculum tubes, also, are prepared in the same way. It is, however, important to incubate the cells at 37° C. for 72 hours. The titration is carried out with 0.1 *N* alkali as usual.

The standard response curve for DL-methionine should run from 0-50 γ and calculations are made as described for the other assays.

5. Glutamic Acid

Organism: Leuconostoc mesenteroides P-60 is recommended for this assay. *Lactobacillus arabinosus* has also been used.

Basal Medium: The medium described in Table IV is used (with the following modifications): L-aspartic acid is added (400 mg./-500 ml.) to the basal medium and the glutamic acid is omitted. The pH is adjusted to 6.4 instead of 6.8. To each assay tube, 100 γ of glutamine are added. The latter is necessary in order to get a curvilinear response.

Preparation of the Inoculum: The inoculum is prepared as for the aspartic acid assay.

Standard Solution of L-Glutamic Acid: The organism will respond well over the range of 0-120 γ of the L-isomer. DL-Glutamic acid should not be used in preparing the standard curve since there is some suggestion that part of the D-enantiomorph is used (Dunn, 1944).

The remainder of the procedure is similar to the other assays. The tubes are incubated at 30° C. for 72 hours.

6. Glycine, Proline, Histidine and Phenylalanine

Organism: Leuconostoc mesenteroides P-60 is employed for these assays.

Basal Medium: The medium given in Table IV is used. The only changes necessary are that 400 mg./500 ml. L-aspartic acid be added and the glycine, histidine or proline omitted.

Standard Solution of the Amino Acids: The range for glycine and proline is 0-50 γ. For histidine the range is 0-25 γ while the range for phenylalanine is 0-50 γ. If DL-phenylalanine is used, however, the range is 0-100 γ of the DL-isomer.

The inoculum and assays are the same as described earlier for assays employing *L. mesenteroides* P-60. The tubes are incubated for 72 hours at 30° C. High blanks may be encountered in the glycine assay if precaution is not taken to use pure amino acids. DL-Alanine has at times been found to contain appreciable amounts of glycine. If such difficulty is encountered, the condition may be remedied as follows. The amino acids going into the basal medium are weighed out and dissolved in water. The pH of the solution is adjusted to 3.0 with 10 N HCl, 2 g. of activated carbon (Darco G-60) are added and the solution is boiled for 30 minutes. The carbon is filtered off and the amino acid solution is then combined with the other components of the medium.

7. Leucine, Isoleucine and Valine

Organism: The organism of choice is *Lactobacillus arabinosus* 17-5. Other organisms have also been used for these assays (*cf.*, Shankman, 1943).

Basal Medium: The medium employed for these assays is essentially that recommended by Schweigert (1944). The composition of the medium is given in Table V.

TABLE V

MEDIUM FOR ASSAY OF ISOLEUCINE, LEUCINE AND VALINE

L-Glutamic acid	0.4 g.	Adenine sulfate•H_2O	10 mg.
DL-Aspartic acid	0.8 g.	Guanine•HCl•$2H_2O$	10 mg.
L-Lysine•HCl	0.2 g.	Uracil	10 mg.
DL-Threonine	0.2 g.	Xanthine	10 mg.
DL-Valine*	0.2 g.	Thiamine•HCl	200 γ
DL-Isoleucine*	0.2 g.	Ca-*d*-pantothenate	200 γ
DL-Alanine	0.2 g.	Pyridoxine•HCl	200 γ
L-Leucine*	0.1 g.	Riboflavin	400 γ
L-Cystine	0.1 g.	Nicotinic acid	400 γ
DL-Methionine	0.1 g.	*p*-Aminobenzoic acid	100 γ
DL-Phenylalanine	0.1 g.	Biotin	0.5 γ
L-Arginine•HCl	50 mg.	Sodium chloride	5 g.
L-Histidine•HCl•H_2O	50 mg.	Ammonium sulfate	3 g.
L-Tyrosine	40 mg.	Salt solution *A*	5 ml.
Glucose	20 g.	Salt solution *B*	5 ml.
Sodium acetate•$3H_2O$	33 g.	Water *q.s.*	500 ml.
		pH 6.8	

* Amino acid to be assayed is omitted from the medium.

Precaution must be taken to use pure amino acids. DL-Leucine is quite often contaminated with isoleucine (Hegsted, 1944) while isoleucine may contain leucine and allo-isoleucine. If difficulty is encountered, the procedure for purification outlined in the previous assay may be employed.

Preparation of the Inoculum: Lactobacillus arabinosus 17-5 is maintained on the following medium: 1% yeast extract, 0.25% Proteose Peptone No. 3 (Difco), 0.5% glucose, 0.2% KH_2PO_4, 0.02% Tween 80 (Atlas Powder Co.), 20 mg. DL-tryptophan and 1.5% agar. The pH is adjusted to 6.8. The inoculum tube is prepared by making a transfer from the agar stab culture to a tube of the basal medium to which 0.5% yeast extract has been added. The culture is incubated at 30° C. for 24 hours. The remainder of the procedure is the same as described in the α-alanine assay.

Standard Solution of the Amino Acids: The effective range for these amino acids is 0-60 γ. If the racemate is used, the range will be 0-120 γ.

The assay procedure is the same as for the others described. Incubation is carried out at 30° C. for 72 hours.

8. Serine

Organism: The organism most frequently used for this assay is *Leuconostoc mesenteroides* P-60.

Basal Medium: The medium for this assay is that given in Table IV with the exception that glycine and serine are omitted and 400 mg. of DL-aspartic acid are added. Although pyridoxal is not required by the organism, it must be completely absent or present in excess if a valid assay is to be realized. For routine assay, therefore, pyridoxal is added in excess to offset any addition from the hydrolyzate. When serine is present in low concentrations, as it would be at the lower end of the response curve, the presence of glycine will stimulate growth if carbon dioxide or leucovorin (folinic acid) is also present (Lascelles, 1954). It is therefore necessary to exclude folinic acid from the medium and to prevent accumulation of carbon dioxide. Folinic acid is destroyed by autoclaving the test substances at 115° C. (10 lbs./sq. in.) for 10 minutes at pH 2.0. This will normally be accomplished if HCl hydrolyzates of the protein are used. Incubation of the assay tubes should be done in a container allowing for the replacement of the atmospheric gases with nitrogen or other inert gas. A vacuum desiccator is suitable for this purpose.

Standard Solution of Serine: The range for L-serine is 0-50 γ and 0-100 γ for the racemate. Acid hydrolyzates are used (*cf.*, Chapter II).

The inoculum is prepared in the same way as for the aspartic acid assay. The general assay procedure (see aspartic acid) is followed.

9. Tryptophan

Organism: Lactobacillus arabinosus 17-5 is generally used for this assay.

Basal Medium: The medium of Barton-Wright (1946) is given in

TABLE VI

Tryptophan Assay Medium

Casein hydrolyzate	10 g.	Pyridoxine•HCl	100 γ
L-Cystine	200 mg.	Riboflavin	200 γ
Glucose	20 g.	*p*-Aminobenzoic acid	100 γ
Sodium acetate•$3H_2O$	33 g.	Nicotinic acid	400 γ
Xylose	1 g.	Biotin	0.5 γ
Adenine sulfate•H_2O	10 mg.	Sodium chloride	5 g.
Guanine•HCl•$2H_2O$	10 mg.	Ammonium sulfate	3 g.
Uracil	10 mg.	Salt solution *A*	5 ml.
Xanthine	10 mg.	Salt solution *B*	5 ml.
Thiamine•HCl	100 γ	Water *q.s.*	500 ml.
Ca-*d*-pantothenate	200 γ	pH 6.8	

Table VI. Some modification has been made on the casein hydrolyzate.

Preparation of the Inoculum: The culture is carried on the stock culture medium described in the leucine assay. The inoculum tube is prepared by transferring from the stock culture to a tube of the tryptophan basal medium to which 20 mg.% DL-tryptophan has previously been added before sterilization. The culture is incubated for 24 hours, at 30° C. The organisms are washed by centrifugation with distilled water as described earlier and then suspended in 100 ml. of distilled water. One drop of this suspension is used to inoculate each assay tube.

Standard Solution of Tryptophan: It is important that L-tryptophan be used for the preparation of the standard since the D-isomer may result in growth inhibition at the lower levels of L-tryptophan (Prescott, 1949). The range for establishing the standard curve is 0-12 γ.

Preparation of the Hydrolyzate: Alkaline hydrolysis is not thoroughly satisfactory since there may be some destruction of tryptophan. For this reason, enzymatic digestion has been used (Woolley, J. G., 1945; *cf.*, Chapter II). The method, with slight modifications, is as follows. To 1 g. of protein add 50 ml. of distilled water and adjust the pH with 6 *N* HCl to 1.5-2.0. To this suspension 10 mg. of pepsin are then added and the material is incubated at 37° C. for 18 hours. At the end of the first incubation period, 3 g. of K_2HPO_4 are added and the pH adjusted to 8.4. Then 10 mg. of trypsin (1:3000) are added and the material is incubated at 40° C. for 24 hours. The pH is adjusted to 7.8 with dilute acid and 100 mg. of erepsin are added. The digest is incubated for 24 hours. After the final digestion period, the pH is adjusted to 6.8 and the volume brought up to 100 ml. The digest is extracted with diethyl ether to remove lipoidal substances as well as traces of anthranilic acid and indole. The digest may be filtered before use if necessary.

Since there will be some liberation of tryptophan, as well as stimulatory peptides (Kizer, 1955), as a result of the autodigestion of the enzymes, it is necessary to determine the degree of stimulation and the amount of tryptophan entering the assay via this route. This can be accomplished by setting up a parallel digestion using ten times as much enzyme and omitting the protein sample. This material is assayed along with the test material and the values obtained from the enzymes are subtracted from the final results. However, there is no reason to believe that the extent and type of autodigestion in the absence of protein is the same as in its presence.

An upward drift in the assay will either indicate incomplete digestion of the sample or the presence of stimulatory substances. A downward drift will indicate the presence of inhibitory substances.

The remainder of the operations for conducting the assay are similar to the others described. The tubes are incubated for 72 hours at 30° C. Titration of the acid is effected with 0.1 N sodium hydroxide.

CALCULATIONS

It will be found that when the response of the organism (acid production) is plotted against the graded amount of the test substance, a curve is obtained. It is the exception in amino acid assays when plots of this type are linear. For some of the vitamins, a straight line is, however, to be expected. It will be found that with the assays described here, plotting dose *versus* response will result in a curvilinear expression. Since it would facilitate matters, so far as the calculations are concerned, if a straight line could be obtained, several attempts have been made toward this end. Wood (1947) was able to transform these curves into linear plots through the use of logarithms. If the log of the response is then plotted against the log of the dose, a fairly good straight line is obtained. When both the standard response and the test response are so plotted, two parallel lines are obtained. For that matter, lack of parallelism over a good portion of the plot indicates the presence of stimulatory or inhibitory substances. Lack of parallelism indicates an invalid assay. It is necessary when setting up the plot, to draw in the straight line showing the best fit.

The method of calculation is a modification of Wood's (1947) procedure. The response values (ordinate) are plotted against the dose (abscissa) on logarithmic paper. Generally, 2 cycle × 2 cycle paper is adequate. A horizontal line is drawn from the test line to the standard line. At the points of intersection on these two lines perpendiculars are dropped to the *x*-axis. The perpendiculars will give the values of the milliliters of test substance and the corresponding micrograms of pure substance respectively. From this, the amount of amino acid can be expressed in any convenient term. Several such readings are made along the parallel portion of the lines. It is advisable to make the measurements at the extreme limits of linearity. If the agreement is good, the mean value is taken as the estimate of the amino acid in the test substance. As Irwin (1943) has pointed out, it is best to ignore those points which fall outside the range of linearity.

In Figure 1 the plot of an assay for L-cystine is presented. If we draw a horizontal line from the test line to the standard line at the lower limit, we observe that 0.3 ml. of test material contains 6 γ of L-cystine and therefore 1 ml. of test material is equivalent to 20 γ of cystine. At the upper limit of linearity we observe that 1.5 ml. of test material corresponds to 30 γ of cystine (or again 20 γ of L-cystine per ml. of test substance). In the case in point, the correlation is excellent; however, if the values fall within 5% of each other they are considered acceptable. For greater precision, Wood (1947) points out that it is necessary to modify the experimental design in such a manner to permit the aliquots of both test and standard material to be added in a geometrical rather than an arithmetical progression. The use of a geometrical progression in the latter case allows for more uniform spacing of the values when plotted logarithmically. It should be pointed out, too, that the ultimate in precision would require the application of statistical analysis to the values so obtained.

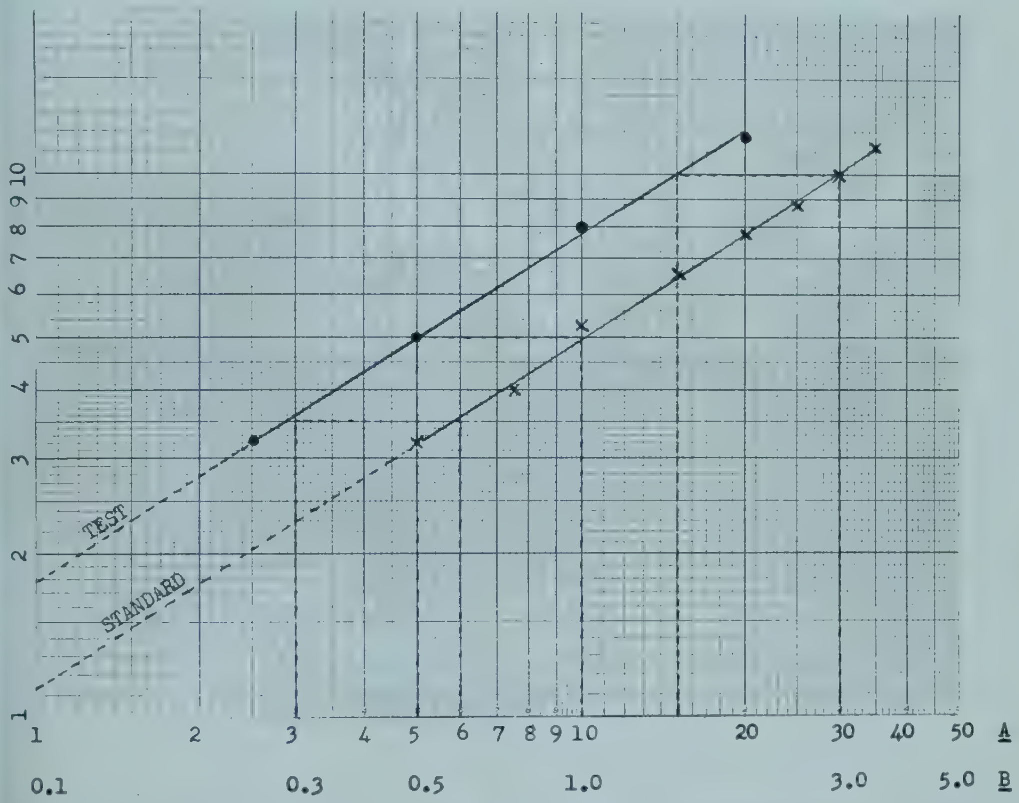

FIGURE 1. Microbiological Assay of L-Cystine with *Leuconostoc mesenteroides* P-60. Horizontal axis *A*: γ of Standard. Horizontal axis *B*: Milliliters of Test Material. Vertical axis: Milliliters of 0.1 *N* Sodium Hydroxide.

The validity of an assay must be established at the outset. One approach is to compare the estimates of the microbiological assay with other methods, *i.e.*, chemical and chromatographic. Another criterion that can be applied is to determine the consistency of the results on the same material on different days and also with different organisms capable of responding to the amino acid (Ågren, 1948). A third criterion is the theoretical recovery of the addition of known amounts of amino acid to the assay.

One final observation must be made in analyzing the results of the assay. It is necessary to examine for any significant "drift." An assay is said to drift if the values obtained from the curve show a regular trend when calculated from different levels. For example, if in the cystine assay shown in Figure 1, the estimates obtained proceeded from 25 γ/ml. to 20 γ/ml. as points were taken successively from the low end of the plot to the high end, this would indicate a downward drift. The presence of any drift, upward or downward, invalidates the assay.

Although the pitfalls may seem abundant, the microbiological assay is still one of our important tools in analytical chemistry and is to be highly recommended.

CHAPTER V

Estimation of Amino Acids in Protein Hydrolyzates by Paper Chromatography

THE METHODS to be described in this Chapter are based on the partition paper chromatography described by Martin, Synge, Consden and Gordon. No attempt will be made to describe all the various modifications of paper chromatographic techniques which have been used to estimate amino acids in protein hydrolyzates as many of these have been summarized in the monograph of Block, Durrum and Zweig (1955). However, two procedures will be described: the first is based upon the direct estimation of amino acids on paper chromatograms employing a densitometer; the second is based upon the separation of the 2,4-dinitrophenyl derivatives of the amino acids by paper chromatography, followed by elution of the yellow derivatives and colorimetric determination of the eluted compound.

ESTIMATION OF AMINO ACIDS BY MAXIMUM COLOR DENSITY ON ONE-DIMENSIONAL PAPER CHROMATOGRAMS

The quantitative estimation of colored substances directly on the paper after one-dimensional paper chromatography is more accurate than on two-dimensional chromatograms, as both the standards and the unknowns are placed on the same sheet. The procedure given in this section is that developed by Block (1950).

1. Acid Hydrolysis for All Amino Acids Except Tryptophan

Unless the test material is limited in quantity, sufficient sample to contain 32.0 mg. of nitrogen (equivalent to 200 mg. of "protein") is hydrolyzed with 20 ml. of 6 *N* HCl under reflux for 18 to 24 hours. At the end of the hydrolysis period, the contents of the hydrolyzing flask are concentrated to dryness on the steam bath.* The hydrolyzate is then dissolved in 25 ml. of water and filtered, and after thorough washing of the precipitate, the solution is decolorized with a little Darco G-60. After removal of the carbon, the hydrolyzate is dissolved in exactly 5.0 ml. of 10% aqueous isopropyl alcohol. The hydrolyzate then contains the equivalent of 40 mg. of protein per milliliter. It may be kept without harm for at least a year at room temperature. Subdilutions containing 20, 10, 5, and 2.5 mg. of protein per milliliter are prepared. Other methods of hydrolysis as described in Chapter II may also be used.

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* The HCl may also be removed by extraction with a 5% solution of methyldioctylamine in chloroform (Smith, E. Lester, 1948).

2. Alkline Hydrolysis for Tryptophan

A sample of protein containing 32.0 mg. of nitrogen is boiled under reflux with 20 ml. of 14% barium hydroxide for 18 to 20 hours. The excess $Ba(OH)_2$ is removed from the diluted hydrolyzate by carbon dioxide (dry ice). The precipitate is washed twice with four times its volume of water, and the supernatant portions are pooled and evaporated to dryness *in vacuo*. The residue is then taken up in exactly 5.0 ml. of 10% isopropyl alcohol.

3. Preparation of Standards

If the amino acid to be used as a standard is suspected of being impure, as shown either chromatographically or chemically, the following procedure is recommended for purification.

Table VII gives a summary of the conditions employed for the purification of amino acids (Dunn, 1947a; Hughes, I. W., 1949; *cf.*, Neuberg, 1951 for resolution of amino acids). Hughes (1949) has reported that impurities up to 1% in amino acid solutions can be easily detected by comparing the vapor pressures of the two solutions. The one contains a slight excess of solid, the other a large excess. The Craig counter-current method (Craig, 1950; Block and Bolling, 1951, pp. 453-464; Kepes, 1953, Kies, 1951) and paper chromatography are, of course, useful in ascertaining impurities in amino acids (*cf.*, Crumpler, 1949; Sulser, 1954a).

After the purity of the amino acids has been ascertained, the standards may be prepared in the following manner. Weigh the indicated quantities of amino acids into a 125-ml. Erlenmeyer flask: alanine, 89 mg.; arginine•HCl, 211 mg.; aspartic acid, 133 mg.; cystine, 240 mg.; glutamic acid, 73.5 mg.; glycine, 75 mg.; histidine•-HCl•H_2O, 210 mg.; hydroxyproline, 131 mg.; isoleucine, 131 mg.; leucine, 131 mg.; lysine•HCl, 183 mg.; methionine, 149 mg.; phenylalanine, 165 mg.; proline, 115 mg.; serine, 105 mg.; threonine, 119

TABLE VII

PURIFICATION OF AMINO ACIDS

Amino Acid	*1st Crystallization*		*2nd Crystallization*		*Wash Fluid*[d]
		ml./g. Amino Acid		*ml./g. Amino Acid*	
DL-Alanine	Water	3.0	95% Ethanol	6.0	95% Ethanol
L-Alanine	Water	3.0	95% Ethanol	6.0	95% Ethanol
L-Arginine	Water	1.2			40-95% Ethanol
L-Arginine•HCl	0.4 N HCl[a]	0.5			80-95% Ethanol
DL-Aspartic Acid	Water	9.5			Ice water
L-Aspartic Acid	Water	25.0			Ice water
L-Cystine	1.5 N HCl	18.0	0.6 N NH_4-OH to pH 5		Hot water
DL-Glutamic Acid•H_2O	Water[b]	9.0			20-50% Ethanol
L-Glutamic Acid	Water	12.0			50-100% Methanol
Glycine	Water	2.3	95% Ethanol	1.0	35-95% Ethanol
L-Histidine	Water	6.0			50-95% Ethanol
L-Histidine•HCl•H_2O	0.2 N HCl[a]	1.0			75-95% Ethanol
L-Hydroxyproline	50% Methanol 50% Ethanol	3.1			Methanol
DL-Isoleucine	Water	17.5	95% Ethanol	4.4	25-95% Ethanol
DL-Leucine	Water	30.0			25-100% Methanol
L-Leucine	Water	24.0	95% Ethanol	24.0	50-95% Ethanol
DL-Lysine•HCl	0.2 N HCl[a]	1.0			60-95% Ethanol
L-Lysine•HCl	0.2 N HCl[a]	1.0			60-95% Ethanol
DL-Methionine	Water	9.0	95% Ethanol	9.0	50-95% Ethanol
L-Methionine	Water	17.0	95% Ethanol	34.0	80% Ethanol
DL-Phenylalanine	Water	23.0	95% Ethanol	15.0	95% Ethanol
L-Proline	Abs.	15.0			Abs. Ethanol
DL-Serine	Water	13.0	95% Ethanol	4.5	30-95% Ethanol
DL-Threonine	Water	2.5	95% Ethanol	20.0	95% Ethanol
L-Tryptophan	65% Ethanol	42.0			95% Ethanol
DL-Tyrosine	Water	220.0			Ice water
L-Tyrosine	Water	200.0			Ice water
DL-Valine	Water	7.0	95% Ethanol	7.0	50-95% Ethanol

[a] HCl is used to depress hydrolysis of the amino acid hydrochloride. L-Lysine•HCl•$2H_2O$ crystallizes from the HCl solution but the water of crystallization is lost at 45° C.

[b] A maximum temperature of 80° C. is employed to minimize the conversion to pyroglutamic acid. The aqueous solution is seeded with DL-glutamic acid•H_2O immediately before adding ethanol to insure crystallization of the stable monohydrate; otherwise, a mixture of the anhydrous and monohydrated form crystallizes.

[c] A mixture containing 75% *n*-propanol and 25% ethanol (95%) may be used.

[d] The first washing is made with the solvent of lowest concentration and the last with that of the highest concentration.

mg.; tyrosine, 181 mg.; and valine, 117 mg. Add 50 ml. of boiling water and then add the least quantity of 6 *N* HCl drop by drop to dissolve the amino acids. Cool the solution, add 10 ml. of isopropyl alcohol and transfer to a 100-ml. volumetric flask. Dilute to volume.

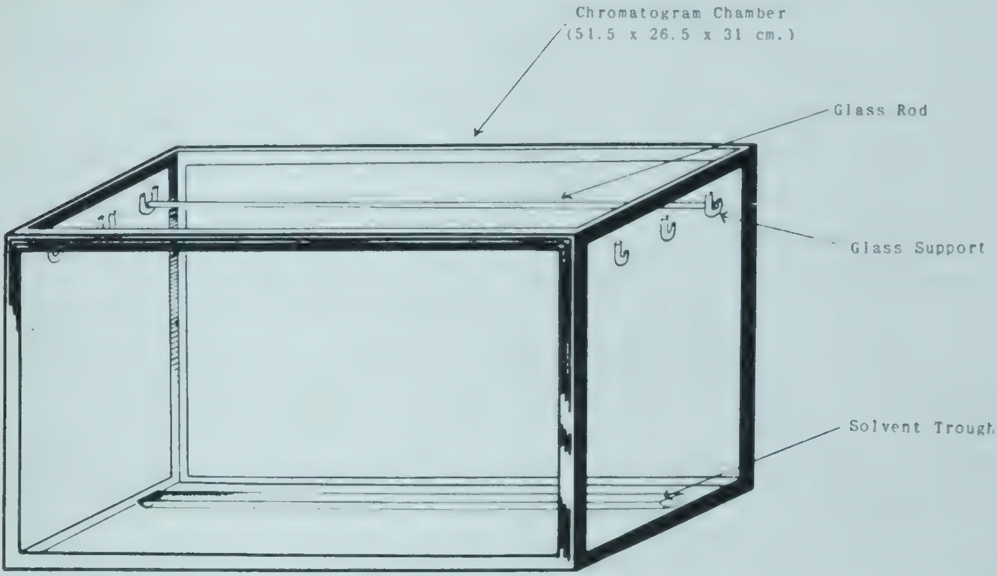

FIGURE 2. Chromatogram chamber for the ascending-descending technique (Block, 1950).

The final concentration of all amino acids except glutamic acid is then 10 μM; glutamic acid is 5 μM. Prepare subdilutions from the 10 μM standard with aqueous 10% isopropyl alcohol to contain 8, 6, 4, 2, 1 and 0.5 μM. Tryptophan standards of the same concentrations should be prepared separately in 10% isopropyl alcohol.

4. Chromatogram Chamber

Ten-gallon rectangular glass aquaria, 52 cm. long, 32 cm. high, and 26 cm. wide, are used. The lower portion of the sides and all the joints are covered by a layer of paraffin. Two glass rods running the length of the chamber are sealed with paraffin to the sides of the chamber about 5 cm. out and 2-3 cm. from the top. A third rod is sealed halfway between the other two at the same height. Glass troughs* are made by sealing the ends of 2-inch borosilicate glass tubing and cutting longitudinally down the middle. (These may also be obtained from the Yonkers Laboratory Supply Company, Yonkers, N. Y.) The chamber is covered with "Saran" wrap and held down with a glass plate (see Figure 2).

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* Stainless steel troughs are preferable.

5. Application of Solutions

Three sheets of filter paper, 18″ x 11″, are cut and marked with a series of marks 2.5 cm. from the bottom of the sheets and 2 cm. apart. This allows 22 spots to be applied to each sheet. The standards at four levels of concentration and the unknowns at two or three levels of concentration are applied with a micropipette. The spots are dried with an infrared lamp or a hair drier. The lower edge of the sheets is immersed in the solvent. A crease is made a few centimeters from the top of the sheet and the paper is supported by the glass rod. A wooden clothespin serves as a convenient counterweight. The chamber is covered with the lid, and the solvent is allowed to travel approximately 23 cm. beyond the point of application of the substances.

6. Replication

Five replicate spots for each level of standard and for the two or more levels of the unknown are usually employed. Each sheet should have at least one application of each level of standard. After development of the color and densitometry, the results are averaged and the concentration of the unknown is read from the standard curve or calculated by the method of least squares. Thus, it is advisable to use the *same* pipette for the application of *both* standards and unknowns.

When several proteins are analyzed simultaneously, it is advisable to stagger the applications of standards and unknowns over the sheet in order to avoid having the same material always located near either end of the sheet.

7. Method of Separating Amino Acids

Because all the amino acids commonly present in a protein hydrolyzate cannot be readily separated on a single one-dimensional chromatogram, they are estimated in groups as follows:

Group A—For the determination of Aspartic Acid, Glutamic Acid,✓ Cystine, Serine, Glycine, Threonine, Alanine.

Paper: Whatman No. 1,[*] 18″ long x 11″ high.

Pipette: 2.5 lambda, or if necessary 1 lambda.

Standards: 1, 2, 4, 6 and 8 μM.

Unknown to Contain at Lowest Level: (Also apply two or three times concentration, and if necessary four times.)

Aspartic Acid	($R_f = 0.08$)	0.20-0.60 mg./ml.
Glutamic Acid	($R_f = 0.16$)	0.20-0.50 mg./ml.
Cystine	($R_f = 0.22$)	0.20-0.50 mg./ml.
Serine	($R_f = 0.32$)	0.20-0.45 mg./ml.
Glycine	($R_f = 0.38$)	0.12-0.30 mg./ml.
Threonine	($R_f = 0.46$)	0.20-0.50 mg./ml.
Alanine	($R_f = 0.55$)	0.14-0.40 mg./ml.

Solvent: 88% liquid phenol:2-propanol:water = 100:20:20 $^{v}/v$. Add 15 mg. of 8-hydroxyquinoline per 100 ml. of solvent. Just before use add 1 ml. of 7.5 N NH_4OH per 100 ml. of solvent.[†] (Note: For best results use Mallinckrodt Brand phenol "Special for Chromatography.") Ordinary phenol may also be purified by distillation over a mixture of aluminum filings and 0.05% sodium bicarbonate (Housewright, 1950). Warm the mixture gently to dissolve the phenol. Treat the hydrolyzate spots with the vapors of 1:4 ammonia for 4 minutes after the spots have dried in order to neutralize any free hydrochloric acid.

Length of Run: Run to 25 cm. Dry in hood and redevelop with solvent.

Color Reagent: 0.25% ninhydrin in acetone $^{w}/v$. Spray or dip the chromatogram into this reagent. Air-dry in the hood for 5 minutes and then heat at 35-40° C. at 80-100% humidity for one hour. Place the chromatograms in the dark and read the next day.

Group B—For the determination of Cystine, Lysine, Histidine, Arginine, Alanine, Tyrosine, Phenylalanine, Isoleucine, Leucine.

Paper: Whatman No. 1, 18″ long x 11″ high.

Pipette: 1 or 2.5 lambda.

Standards: 1, 2, 4, 6 and 8 μM.

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[*] Tighter spots are obtained with paper washed with 2 N acetic acid, water, 0.5 N LiOH, water, 0.1% $^{w}/v$ calcium acetate, water, 95% $^{v}/v$ ethanol (Connell, 1955).

[†] Unless the ammonia concentration is exactly right, the glutamic acid will divide into two spots above and below cystine. In this case it is preferable to use the following solvent for glutamic acid: 88% liquid phenol:water = 100:20 $^{v}/v$ (8 HO•quinoline). Beakers containing 10 ml. of 1% sodium cyanide and 25 ml. of 3% ammonia respectively, are placed in the chromatogram chamber.

Unknown to Contain at Lowest Level: (Also apply at two or three times the concentration.)

Cystine	(R_A[*] = 0.31)	0.20-0.50 mg./ml.
Lysine	(R_A = 0.42)	0.25-0.60 mg./ml.
Histidine	(R_A = 0.47)	0.25-0.60 mg./ml.
Arginine	(R_A = 0.53)	0.25-0.70 mg./ml.
Alanine	(R_A = 1.00)	0.14-0.40 mg./ml.
Tyrosine	(R_A = 1.15)	0.25-0.65 mg./ml.
Phenylalanine[†]	(R_A = 1.50)	0.30-0.70 mg./ml.
Isoleucine[†]	(R_A = 1.58)	0.30-0.55 mg./ml.
Leucine	(R_A = 1.61)	0.30-0.55 mg./ml.

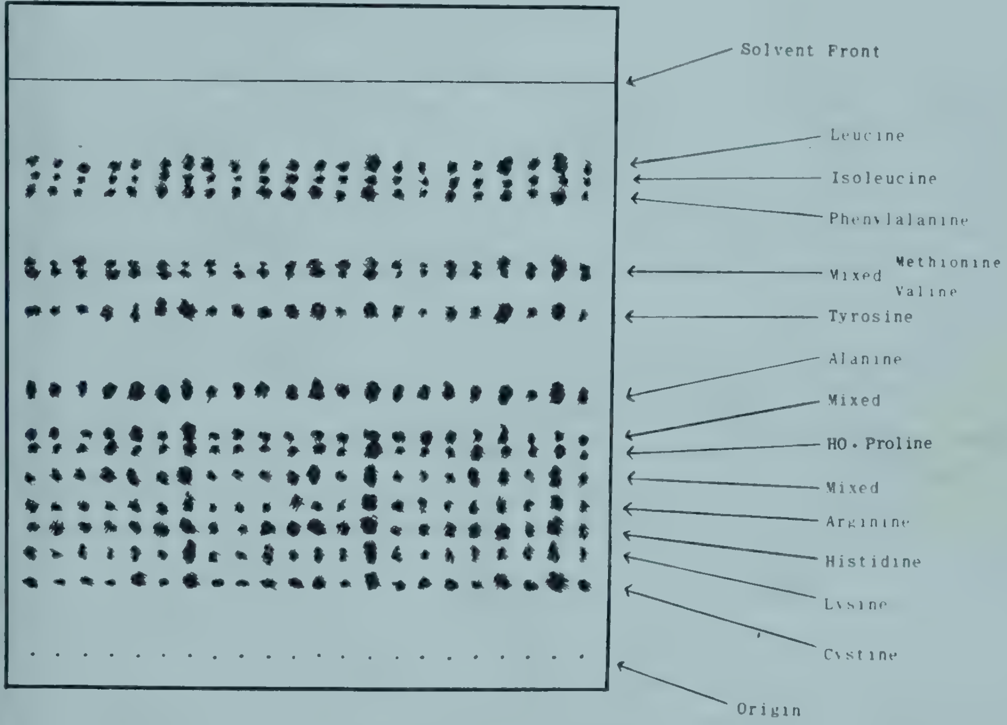
Solvent Front
Leucine
Isoleucine
Phenylalanine
Mixed
Methionine
Valine
Tyrosine
Alanine
Mixed
HO · Proline
Mixed
Arginine
Histidine
Lysine
Cystine
Origin

FIGURE 3. Schematic chromatogram of GROUP B.

Solvent: n-Butyl alcohol:acetic acid:water = 250:60:250 ^{v}/v. Use upper layer after separation. Beakers containing the aqueous phase (lower layer) are placed in the chamber.

Length of Run: Run to 25 cm. Dry in the hood for 20-30 minutes. Repeat this entire procedure for a total of 3 or 4 times.

Color Reagent: 0.25% ninhydrin in acetone. Develop as in *Group A.*

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[*] R_A indicates length of travel relative to that of alanine, which has been assigned the value of 1.00.

[†] These amino acids are best separated by conducting the development at approximately 30° C.

Group C—For the determination of Valine, Methionine, Tyrosine, Isoleucine, Leucine, Phenylalanine.

Paper: Whatman No. 1, 18″ long x 11″ high.

Pipette: 1 or 2.5 lambda.

Standards: 2, 4, 6 and 8 μM.

Unknown to Contain at Lowest Level:

Valine	(R_V* = 1.00)	0.25-0.50 mg./ml.
Methionine	(R_V = 1.10)	0.30-0.65 mg./ml.
Tyrosine	(R_V = 1.18)	0.25-0.65 mg./ml.
Isoleucine	(R_V = 1.25)	0.30-0.55 mg./ml.
Leucine	(R_V = 1.31)	0.30-0.55 mg./ml.
Phenylalanine	(R_V = 1.36)	0.30-0.70 mg./ml.

Solvent: Isoamyl alcohol:pyridine:water:diethylamine — 50:50:35:2 ^{v}/v. Prepare fresh before use. Equilibrate the papers with the solvent. Beakers containing 5 ml. of 1% sodium cyanide and aqueous phenol are also placed in the chamber.

Length of Run: Run to 25 cm. Dry and re-run twice for 25 cm.

Color Reagent: 0.25% ninhydrin in acetone. Develop as in *Group A*.

Group D—For the determination of Histidine and Tyrosine.

Paper: Schleicher and Schuell No. 598, 18″ long x 11″ high.

Pipette: 5 lambda, or if necessary 2.5 lambda.

Standards: 2, 4, 6 and 8 μM.

Unknown to Contain at Lowest Level:

Histidine	(R_f = 0.17)	0.30-0.70 mg./ml.
Tyrosine	(R_f = 0.40)	0.20-0.70 mg./ml.

Solvent: *n*-Butyl alcohol:acetic acid:water. (See *Group B.*)

Length of Run: 25 cm. Repeat once or twice.

Color Reagent: Diazotized sulfanilamide: 1% sulfanilamide in 10% ^{v}/v HCl. Place 5 ml. of sulfanilamide and 5 ml. of 5% $NaNO_2$ in a separatory funnel. Mix for 1 minute. Then add 50 ml. *n*-butyl alcohol. Shake for 1 minute and let stand for 4 minutes. Decant the butyl alcohol layer (containing the diazotized sulfanilamide) and use to spray or dip the chromatogram. Dry the sheet in a current of air in the hood and then spray with half-saturated sodium carbonate. Imidazoles give a deep cherry red color; phenols give a red color.

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* R_V indicates distance of travel relative to that of valine which has been assigned the value of 1.00.

Group E—For the determination of Valine.

Paper: Whatman No. 1, 18″ long x 11″ high.

Pipette: 1 or 2.5 lambda.

Standards: 1, 2, 4 and 6 μM.

Unknown to Contain at Lowest Level:

Valine	($R_f = 0.36$)	0.15-0.50 mg./ml.

Solvent: Lutidine:collidine:water = 1:1:1 v/v. Solvent should also contain 1% diethylamine by volume. Beakers containing 5 ml. of 1% sodium cyanide and 5 ml. of aqueous phenol are placed in the chromatogram chamber. Treat hydrolyzate spots with the vapors of 1:4 ammonia for 4 minutes.

Length of Run: 25 cm.

Color Reagent: 0.25% ninhydrin in acetone. Develop as in *Group A.*

Group F—For the determination of Methionine.

Paper: Schleicher and Schuell No. 598, 18″ long x 11″ high.

Pipette: 5 lambda, or if necessary 2.5 lambda.

Standards: 2, 4, 6 and 8 μM.

Unknown to Contain at Lowest Level:

Methionine	($R_f = 0.50$)	0.30-0.60 mg./ml.

Solvent: n-Butyl alcohol:acetic acid:water. (See *Group B.*)

Length of Run: 25 m.

Color Reagent: Platinic iodide in acetone. Add the following in order: 4 ml. of 0.002 *M* PtK_2Cl_6 (potassium chloroplatinate), 0.25 ml. of 1 *N* KI, 0.4 ml. of 2 *N* HCl and 76 ml. of acetone.* Dip the dried chromatograms in this reagent. Cystine, cysteine, methionine, and some other reducing substances give a white spot on a red-purple background.

Group G—For the determination of Proline and Hydroxyproline.

Paper: Whatman No. 1, 18″ long x 11″ high.

Pipette: 5 lambda.

Standards: 1, 2, 4 and 6 μM.

Unknown to Contain at Lowest Level:

Hydroxyproline	($R_f = 0.20$)	0.25-0.60 mg./ml.
Proline	($R_f = 0.30$)	0.10-0.30 mg./ml.

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* The acetone must be purified by refluxing and distilling over $KMnO_4$ and K_2CO_3.

Solvent: n-Butyl alcohol:acetic acid:water. (See *Group B.*)

Length of Run: 25 cm. for proline only; develop twice for proline *and* hydroxyproline.

Color Reagents: For *proline:* 0.2% isatin in acetone. After spraying chromatogram, air-dry and then put sheet in oven for 10 minutes at 70-76° C. (Oven should be saturated with water vapor.) Read the quantity of proline with the densitometer. After reading the proline

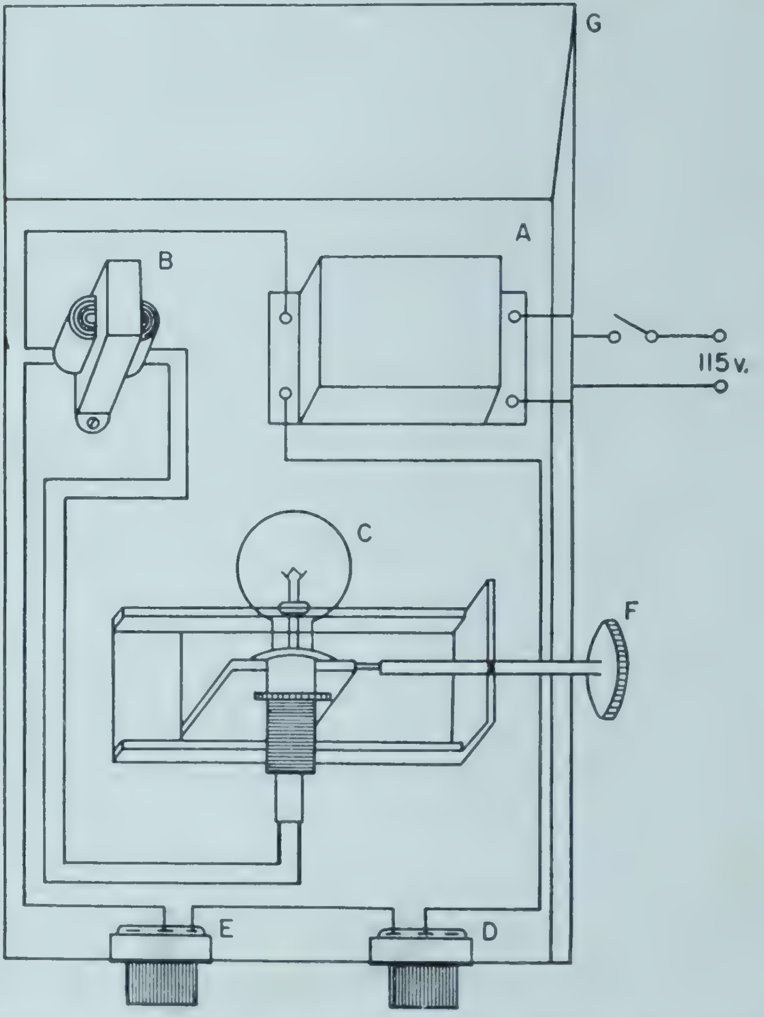

FIGURE 4. Diagram of light source of photoelectric densitometer (Block, 1950).

A. Voltage stabilizer (output 115 volts).
B. Stepdown transformer (115-6 volts).
C. Light source.
D. Light intensity control (coarse).
E. Light intensity control (fine).
F. Control to position light source.
G. Housing for assembly.

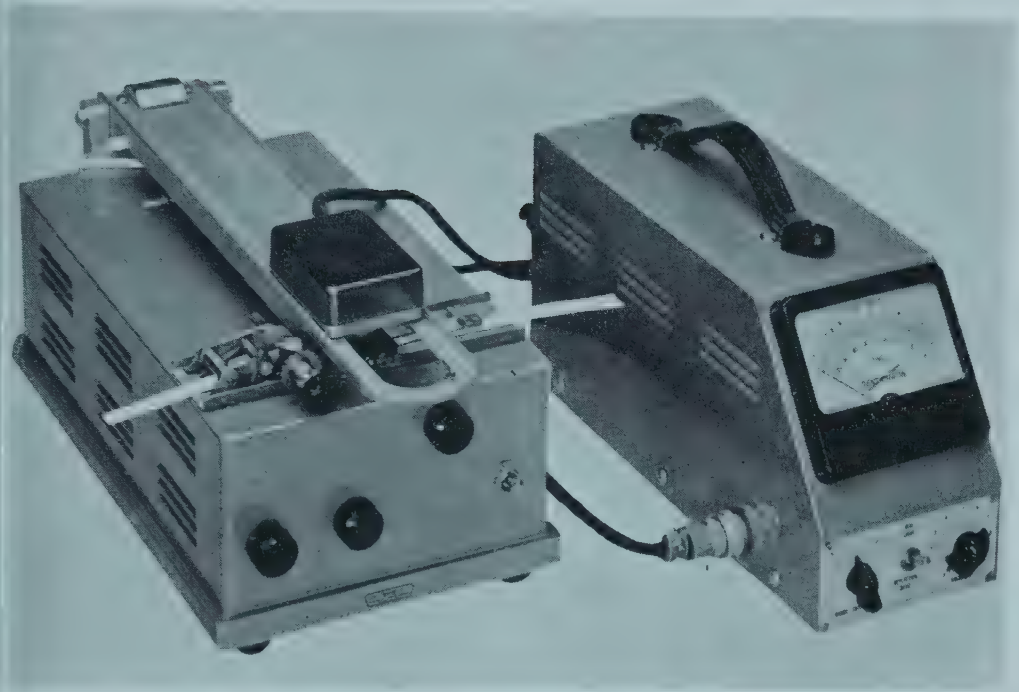

FIGURE 5. Photoelectric Densitometer (Welch Scientific Company).

spots, for *hydroxyproline:* Spray the isatin-developed sheet with a freshly prepared solution of 1 g. of *p*-dimethylaminobenzaldehyde, 90 ml. of acetone and 10 ml. of concentrated HCl. Air-dry in the hood for 30 minutes and read the hydroxyproline spots.

Group H—For the determination of Tryptophan.

Paper: Whatman No. 1, 18″ long x 11″ high.

Pipette: 2.5 or 5 lambda.

Standards: 2, 4, 6 and 8 μM.

Unknown to Contain at Lowest Level:

Tryptophan	($R_f = 0.60$)	0.25-0.80 mg./ml.

Solvent: Isopropyl alcohol:ammonium hydroxide:water = 80:5:15 $^{v}/v$, or 2-butanol:3% ammonia = 150:50 $^{v}/v$.

Length of Run: 25 cm.

Color Reagent: *p*-Dimethylaminobenzaldehyde, develop as in *Group G.*

8. Densitometers

A densitometer consisting of a barrier layer photocell (General Electric Photovoltaic Cell No. 8PV1), a suitable light source, and a sensitive galvanometer (Rubicon Spotlight No. 3402-HH or Pfaltz and Bauer Multiple Mirror Galvanometer No. 1810) is easily constructed. Figure 4 shows the light box in greater detail. The *a/c* line current (115 volts) is passed through a voltage regulator, adjusted by a coarse and fine variable resistance, and is reduced to 6 volts by a stepdown transformer. The line is then connected to the light source, an automobile headlight, whose beam is adjusted by a focusing device to pass through the circular opening. (A light source sold by the Photovolt Corporation may be substituted.) Commercial photoelectric transmission densitometers are manufactured by the W. M. Welch Mfg. Co., Chicago, Ill.; The Photovolt Corporation, New York, N. Y.; Elphorecorder, automatic scanner and integrator, C. A. Brinkmann and Co., Great Neck, L. I., N. Y., and other firms (see Figures 5 and 6).

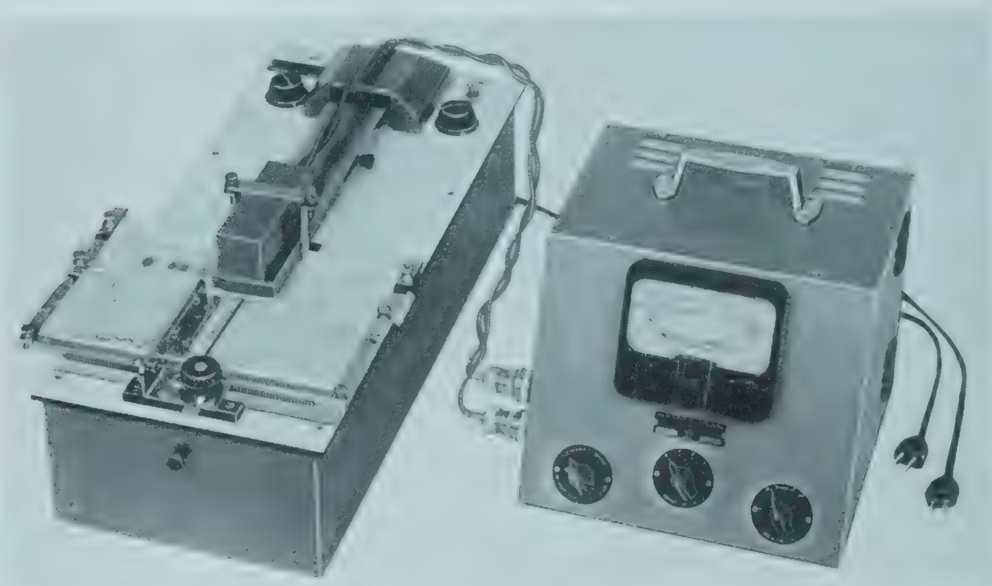

FIGURE 6. Photoelectric Densitometer (Photovolt Corporation).

Any electric recorder that has full-scale excursion for 200 microamperes or less and an input resistance of not more than 2500 ohms can be attached to the densitometer for automatic scanning of the chromatograms (*cf.*, Husband, 1954; Block, Durrum and Zweig, 1955). If Beckman or Coleman Junior spectrophotometers are available, the colored spots may be cut out of the chromatogram, pasted or fastened on the cuvettes with Scotch tape and the color density read (Ehrmantraut, 1954; Schulz, 1954).

9. Method of Reading

All amino acids are roughly outlined in pencil using transmitted light (*x*-ray viewer). This facilitates centering each spot on the densitometer for the determination of the maximum color density. The color density of each amino acid (standard and unknown) is read and the average values of the replicates is calculated. After the hydrolyzates have been prepared and when six or more proteins are run simultaneously, it takes 15-20 minutes of bench time per amino acid per sample.

10. Calculations

The average densitometric readings of the standard[*] solutions may be used to prepare standard curves (see Figure 7) from which the unknowns are read in the usual fashion. The percent transmission is plotted on the abscissa and the concentration in millimoles per liter is plotted on the ordinate of semilogarithmic paper (Keuffel and Esser No. 359-63).

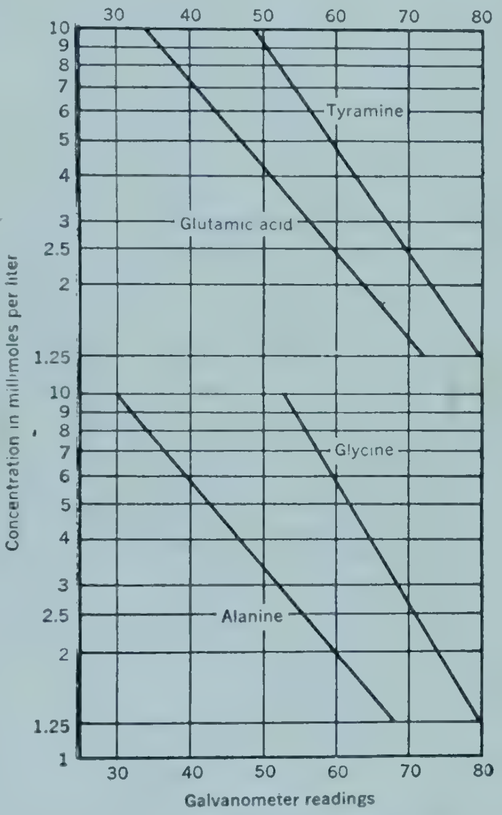

FIGURE 7. Typical calibration curve of several amino acids by the maximum density method.

The results may also be calculated as follows. Assuming that a logarithmic relationship holds between a standard (amino acid) at a single (s) and double level ($2s$) and an unknown at a single (u) and a double level ($2u$), a general equation has been derived which permits calculation of the quantity of unknown, given the average densitometric readings for both standards and unknowns. This method of computation compares very well with the commonly used standard curve procedure and involves the derivation of nine sums which are then substituted in the appropriate equation to solve for the antilog of the lowest unknown level.

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[*] It is important that standards be run each time as the amount of color developed by the ninhydrin will vary unless all conditions of humidity, temperature, time exposure to light, etc., are absolutely constant during the color development.

Derivation of the Sums and Solution of the Equation. Using the average chromatogram readings (as percent transmission) for standard (s and $2s$), and similar levels (u and $2u$) for the unknown, the following nine sums are calculated:

$$s + u, \tag{1}$$
$$(\log s)\ (s + u), \tag{2}$$
$$2s + 2u, \tag{3}$$
$$(\log 2s)\ (2s + 2u), \tag{4}$$
$$s + 2s = Ys, \tag{5}$$
$$u + 2u = Yu, \tag{6}$$
$$\frac{Ys - Yu}{2} = C, \tag{7}$$
$$Ys + Yu = B, \tag{8}$$
$$\log s(s + u) + \log 2s(2s + 2u) = A \tag{9}$$

The values A, B, and C are then substituted in the equation given below:

$$\text{Answer} = \text{Antilog}\ \frac{0.0906C}{(0.1505 + \log s)B - A}.$$

The resulting answer will be related to the weight of the standard (*i.e.*, milligrams, etc.).

Example. In the determination of lysine by paper chromatography, the following data were obtained:

Standard = s = 2 mM.

Unknown = u = 5 mg. protein/ml.

Average % transmission values (from densitometer):

Reading of lower standard = S = 69.4
Reading of higher standard = $2S$ = 56.8
Reading of lower unknown = U = 60.1
Reading of higher unknown = $2U$ = 45.3

From the above figures, the following sums were calculated:

$$S + U = 69.4 + 60.1 = 129.5, \tag{1}$$
$$\log s(S + U) = 0.301 \times 129.5 = 38.98, \tag{2}$$
$$2S + 2U = 56.8 + 45.3 = 102.1, \tag{3}$$
$$(\log 2s)\ (2S + 2U) = 0.602 \times 102.1 = 61.46, \tag{4}$$
$$S + 2S = 69.4 + 56.8 = 126.2 = Ys, \tag{5}$$
$$U + 2U = 60.1 + 45.3 = 105.4 = Yu, \tag{6}$$
$$\frac{Ys - Yu}{2} = \frac{126.2 - 105.4}{2} = +10.4 = C, \tag{7}$$
$$Ys + Yu = 231.6 = B, \tag{8}$$
$$(\log s)\ (S + U) + (\log 2s)\ (2S + 2U) = 38.97 + 61.46 = 100.4 = A. \tag{9}$$

The values A, B, and C are substituted in the appropriate equation which is then solved, and the resulting quantity is used in the calculation of the percent lysine in the protein.

a. Since the lower level of the standard is 2 mM., the appropriate equation is:

$$\text{Antilog}\ \frac{0.0906C}{(0.1505 + \log s)B - A} = \text{Antilog}\ \frac{0.0906C}{(0.1505 + 0.301)B - A}$$
$$= \text{Antilog}\ \frac{0.4515\ B - A}{0.0906C}.$$

b. Substitute values *A*, *B*, and *C*:

$$\text{Antilog}\ \frac{0.0906 \times 10.4}{0.4515 \times 231.6 - 100.4} = \text{Antilog}\ \frac{0.9422}{4.17}$$
$$= \text{Antilog}\ 0.2259 = 1.68\ \text{mM}.$$

c. Total millimoles = 2 × 1.68 = 3.36.

d. To calculate the percent lysine in the protein:

$$\%\ \text{lysine} = \frac{\text{Total millimoles} \times \text{M.W. lysine}}{\text{Mg. protein/ml. (lower level)}} = \frac{3.36 \times 146.19}{5} = 9.82.$$

11. Comments on the Maximum Color Density Method

McFarren (1952) using unidimensional chromatography and the maximum color density method with the Photovolt Densitometer (filter 570 mμ), has carried out a complete amino acid analysis of β-lactoglobulin which compares very favorably with microbiological and starch column analyses of the same preparation. The same group (McFarren, 1951) has reported that the error of the maximum color density method as applied to sugars is from 1 to 5%. Patton (1951), Polson (1951), Gassner (1952), Salander (1953), Kosikowsky (1954) and Bullock (1955) have also reported that accurate analyses of amino acids separated on unidimensional chromatograms are obtained by the maximum color density method. In fact, Miettinen (1953) has reported that replicate determinations on the same strip differ by approximately ± 0.5% for amino acids determined by this method. However, amino acid determinations of replicate chromatograms from the same sample differ 5-10% (*cf.*, Vaeck, 1955). He pointed out that the most important limiting factor is, without doubt, the variation in the chromatograms. Miettinen also mentions that the maximum density method gives practically the same accuracy as scanning the entire spot.

Roland (1954) and White (1953) have also used the maximum color density method on unidimensional chromatograms.* All amino acids except lysine, glutamic acid, and glycine were determined within confidence limits of $P = 0.95$. Roland states: "The procedures . . . provide a less tedious, more rapid, and reasonably accurate approach to the analysis of protein hydrolyzates than has previously been available."

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* Sulser (1954b) reported increased accuracy when a reflectance colorimeter (Lange "Leucometer") was employed.

TABLE VIII

APPROXIMATE AMINO ACID COMPOSITION OF CRYSTALLINE β-LACTOGLOBULIN

(Calculated in grams of amino acid per 16.0 g. of nitrogen)

Arginine	3.1	2.6	3.0	3.0	3.0	3.1
Histidine	2.1	1.0	1.7	1.6	1.7	1.7
Lysine	12.1	11.5	11.5	11.7	12.9	12.1
Tyrosine	3.9	4.0	4.0	3.9	3.7	3.9
Tryptophan	2.5	1.9	—	2.0	—	2.7
Phenylalanine	4.3	3.5	3.7	3.6	3.9	3.8
Cystine	3.5	3.2	—	3.5	—	2.6
Methionine	3.6	3.0	3.2	3.3	3.3	3.7
Serine	4.7	3.4	3.9	5.1	4.0	5.3
Threonine	5.8	4.7	5.3	5.9	5.0	4.9
Leucine	16.0	11.0	15.6	16.0	15.9	16.1
Isoleucine	8.3	5.2	7.5	8.6	6.0	9.3
Valine	5.8	4.6	6.3	5.9	5.8	5.8
Glycine	1.5	1.4	1.4	1.4	1.4	1.7
Alanine	7.0	6.5	6.0	7.3	7.3	7.0
Glutamic acid	17.8	20.2	18.9	20.0	19.5	20.8
Aspartic acid	14.0	11.4	11.6	11.7	11.8	11.5
Proline	5.0	5.0	5.1	5.4	5.3	5.9
Hydroxyproline	0.0	0.0	—	0.0	—	—
NH_3–N	8.5					
N_2 accounted for	100%			99%	100%	103%
Method	Maximum color density		Microbiological and/or chemical		Column chromatography	

It appears that the maximum color density method should be applicable to all types of substances that are separated on one- and two-dimensional paper chromatograms and are colored or give colored derivatives. The most accurate results are obtained when the colored spots are small and either round or slightly elliptical. In order to achieve this it is important that the solution be applied as a round spot of approximately 5 mm. diameter or less. When one-dimensional chromatograms are used, the strip of paper must be wide enough to allow a margin of at least 2 cm. from the edge of the paper to the nearest point on the circumference of the developed spot.

The maximum density of color in a spot, if the spot spreads by diffusion, should be proportional to the amount of substance originally put on the chromatogram (Brimley, 1953). Even if this proposition does not hold under practical conditions, quite accurate estimations have been made from calibration curves which may not agree with any theory. Among the possible causes of departure from theory are the difficulty of determining the maximum color density at a point, *i.e.*, not over a finite area, the variability of the color (especially in the case of ninhydrin), and the possibility of incomplete reaction.

Table VIII illustrates a comparison of the amino acid values of β-lactoglobulin estimated by paper chromatography (maximum color density method), chemical, microbiological, and column chromatographic methods (from Block, 1955). It is apparent that the four procedures give approximately the same results, although for certain amino acids one method may be superior to the others. Table IX compares the calculated amino acid composition of insulin assuming that Sanger's formula is correct with the better amino acid values reported in the literature (after Tristram, 1953).

TABLE IX

AMINO ACID COMPOSITION OF INSULIN
CALCULATED AND FOUND

Amino Acid	*Molar Ratio*	*Calculated Composition* %	*Amino Acid Values from the Literature* %	*Maximum Deviation* %
Arginine	1	3.03	2.5-3.4	18
Histidine	2	5.40	4.9-5.3	9
Lysine	1	2.55	2.4-2.7	6
Tyrosine	4	12.62	12.1-13.0	4
Tryptophan	0	0.0	0.0	0
Phenylalanine	3	8.63	7.9-8.3	9
Cystine	6	12.55	11.2-12.5	11
Methionine	0	0.0	0.0	0
Serine	3	5.54	5.2-6.6	19
Threonine	1	2.07	2.1-3.5	69
Leucine	6	13.70	13.0-14.6	7
Isoleucine	1	2.28	1.6-2.9	30
Valine	5	10.20	7.4-9.1	28
Glycine	4	5.23	4.3-4.6	18
Alanine	3	4.65	4.5-4.6	3
Glutamic acid	7	17.94	18.6-20.2	13
Aspartic acid	3	6.96	5.5-6.9	21
Proline	1	2.00	2.0-2.9	45
Hydroxyproline	0	0.0	0.0	0
Average Deviation				16.3

Table X illustrates the percent deviation from the mean of amino acid values on two separate determinations of β-lactoglobulin (Block, 1955). Here again the error is of the same order of magnitude as that found by the other widely used methods of amino acid analysis (Block and Bolling, 1951).

12. Determination of Optical Configuration of Amino Acids

In this method (Block, Durrum and Zweig, pp. 101-102; Bonetti, 1954) the amino acids are developed on one-dimensional paper chromatograms. After removal of the solvent, the chromatogram is sprayed with a weak solution of D-amino oxidase from the Viobin Corporation, Monticello, Ill., or prepared from sheep's kidney (Negelein, 1939). The chromatogram is then incubated in a moist chamber for several hours, dried, and sprayed with ninhydrin. A decrease in color compared to an untreated chromatogram indicates the presence and amount of D-amino acid. Likewise, the L-amino acid may be detected by destruction with snake venom oxidase (Zeller, 1944).

TABLE X

Percent Deviation From Mean of Amino Acid Values on Two Separate Determinations of β-Lactoglobulin Amino Acids
(Calculated in grams per 16.0 g. of nitrogen)

Amino Acid	*A*	*B*	*Deviation, %*
Arginine	3.2	3.0	3
Histidine	2.1	2.1	0
Lysine	11.5	12.6	5
Tyrosine	3.9	3.9	0
Phenylalanine	4.2	4.3	1
Cystine	3.2	3.8	9
Methionine	3.6	3.6	0
Serine	4.7	4.7	0
Threonine	5.4	6.2	7
Leucine	16.0	—	—
Isoleucine	8.3	8.5	1
Valine	6.0	5.5	5
Glycine	1.5	1.5	0
Alanine	6.8	7.1	2
Glutamic acid	17.8	17.8	0
Aspartic acid	—	14.0	—
Proline	4.8	5.2	4
Average Deviation			3

13. General Comments

Other methods for the estimation of amino acids on one- and two-dimensional chromatograms and the general technique of paper chromatography are described by Akerfeldt (1954), Berry (1949), Block, Durrum and Zweig (1955), Bode (1952), Boissonnas (1950), Brown, G. L. (1953), Cadavieco (1954), Campbell (1954), Chandler (1955), Chibnall (1954), Connell (1955), Darmon (1954), Dent

(1954a), de Wael (1954), Ehrmantraut (1954), Fischer (1953b), Gerok (1955), Husband (1954), Keil (1954a), Kofrányi (1955), Krishnamurthy (1955), Paskhina (1954), Patton (1954), Pernis (1953), Polson (1951), Rangneker (1954), Redfield (1952), Salander (1953), Schlögl (1953), Schulz (1954), Smith, A. M. (1951b), Sulser (1953, 1954ab), Turba (1954ab), Underwood, J. C. (1953), Wellington (1951, 1952), Williams, R. J. (1951) and Wunderly (1954a).

Grassmann (1955) recommends the following procedure for the estimation of amino acids on one-dimensional chromatograms. This is a modification of the original procedure of Block (1948) and Bull (1949).

a. The protein hydrolyzate is separated into five fractions by continuous paper electrophoresis (*cf*., Block, Durrum and Zweig, 1955). These are cysteic acid, aspartic acid, glutamic acid, neutral amino acids and basic amino acids. The first three amino acids are determined directly from the elutriates.

b. The neutral amino acids are separated by paper chromatography using phenol:cresol = 1:1 v/v saturated with water and with *n*-butyl alcohol:acetic acid:water = 4:1:5 v/v on Schleicher and Schuell paper No. 2043M. The amino acids are *streaked* rather than spotted on the paper.

c. The three basic amino acids are separated by paper chromatography using the phenol:cresol solvent in the presence of ammonium hydroxide.

d. After removal of the solvent, the colors are developed with 1% ninhydrin in *n*-butyl alcohol:phenol = 90:10 v/v. The color is brought out by heating in a steamed cabinet at 100° C. for 3 minutes and dried in an oven at 50° C.

e. The chromatograms are made transparent by immersion in mineral oil (*cf*., Block, Durrum and Zweig, 1955) and the entire chromatogram is scanned in a photoelectric densitometer using a 546 mμ light source. Standard curves are prepared for each amino acid by plotting the area times the distance traveled in millimeters against the concentration.

The error is in the order of ± 3% when four replicated chromatograms are carried out. The useful range is from 0.05 to 0.30 micromoles of amino acid.

14. Useful Solvents

Some of the many organic solvents which have been used to separate amino acids have been given earlier. Other useful ones from Block, Durrum and Zweig (1955) are given below.

1) Acetone:urea:water = 60:0.5:40 v/w/v.
2) *tert*-Amyl alcohol saturated with water.
3) *tert*-Amyl alcohol:*sec*-butyl alcohol:water = 4:4:1.
4) *n*-Butyl alcohol:acetic acid:glycerol:water = 830:25:26:120, prepared fresh; HCN atmosphere.
5) *n*-Butyl alcohol:acetic acid:water = 250:60:250 v/v/v. Store in the presence of aqueous layer.
6) *n*-Butyl alcohol:acetic acid:water = 40:10:50.
7) *n*-Butyl alcohol:acetic acid:water = 68:5:27.
8) *n*-Butyl alcohol:acetic acid:water = 4:1:1.
9) *n*-Butyl alcohol:2 *N* acetic acid = 1:1; for tyrosine, diiodotyrosine, etc.
10) *n*-Butyl alcohol:2 *N* ammonia:dioxane = 4:5:1 v/v; for tyrosine, diiodotyrosine, etc.
11) *n*-Butyl alcohol saturated with 2 *N* NH_4OH.
12) *n*-Butyl alcohol:benzyl alcohol = 1:1 v/v, saturated with pH 8.4 buffer (50 ml. of 0.067 *M* boric acid and KCl + 8.55 ml. of 0.067 *M* NaOH); for leucine and isoleucine.
13) *n*-Butyl alcohol:benzyl alcohol = 1:1, saturated with water, in diethylamine atmosphere.
14) *n*-Butyl alcohol:*tert*-butyl alcohol:0.1 *N* HCl = 3:1:1.
15) *n*-Butyl alcohol:dioxane = 4:1. The dioxane is saturated with 2 *N* NH_4OH. Used for thyroxine, etc.
16) *n*-Butyl alcohol:dioxane = 4:1, saturated with water.
17) *n*-Butyl alcohol:ethyl alcohol:acetic acid:water = 4:1:1:10.
18) *n*-Butyl alcohol:formic acid:water = 600:50:50. Reflux for 1 hour, then add 300 ml. of water to hot solution, shake, and age for 24 hours. Use in place of phenol.
19) *n*-Butyl alcohol, saturated with 2 *N* formic acid; for thyroxine, etc.
20) *n*-Butyl alcohol:glycol monochlorohydrin:ammonia:water = 50:10:5:16.
21) *n*-Butyl alcohol:*n*-propyl alcohol:0.1 *N* HCl = 1:1:1.
22) *n*-Butyl alcohol:*n*-propyl alcohol:0.1 *N* HCl = 1:1:1 in 0.3% ammonia atmosphere.
23) *n*-Butyl alcohol:pyridine:water = 4:5:1.
24) *n*-Butyl alcohol:pyridine:water = 1:1:1; for pipecolic acid.
25) *n*-Butyl alcohol:water = 85:15 v/v.
26) *sec*-Butyl alcohol:acetic acid:water = 33:2:22; for thyroxine, diiodotyrosine, etc.
27) *sec*-Butyl alcohol:3% ammonia = 90:40
28) *sec*-Butyl alcohol:3% ammonia = 3:1.

29) *sec*-Butyl alcohol:3% ammonia = 150:60. Run twice.
30) *sec*-Butyl alcohol:*tert*-butyl alcohol:water = 4:1:3 in 6 *N* HCl or 0.3% ammonia atmosphere.
31) *sec*-Butyl alcohol:formic acid:water = 150:30:20.
32) *tert*-Butyl alcohol:ethylmethyl ketone:formic acid:water = 160:160:1:39 v/v/v; especially useful for separating leucine and isoleucine.
33) *tert*-Butyl alcohol:ethylmethyl ketone:diethylamine = 40:40:-20:4.
34) *tert*-Butyl alcohol:formic acid:water = 70:15:15 v/v/v.
35) Collidine (buffered). Saturate with pH 9.0 buffer (50 ml. of 0.067 *M* boric acid in 0.067 *M* KCl + 21.30 ml. of 0.067 *M* NaOH).
36) Collidine:water = 125:44 v/v. Run in concentrated NH_4OH atmosphere.
37) *m*-Cresol (buffered), saturated with pH 8.4 buffer (50 ml. of 0.067 *M* boric acid in 0.067 *M* KCl + 8.55 ml. of 0.067 *M* NaOH.
38) *m*-Cresol, saturated with water. It may be used alone in HCN and 0.3% ammonia, or in 0.03% ammonia atmosphere. Cresol is especially valuable for the smaller peptides.
39) 70% Ethanol.
40) 77% Ethanol or with 1% diethylamine.
41) 80% Ethanol:H_2SO_4 = 1000:5 v/v; for tryptophan, kynurenine, etc.
42) Absolute ethyl alcohol:*tert*-butanol:ammonia:water = 60:20:-5:15.
43) Ethyl alcohol:water:urea = 80:20:0.5 v/v/w; especially useful for aspartic acid and glutamic acid.
44) 2,6-Lutidine[*]:*tert*-amyl alcohol:water = 55:20:25 + 1-2% diethylamine.
45) 2,6-Lutidine:*tert*-amyl alcohol = 1:1 v/v; then saturate with water and add 0.1% diethylamine.
46) 2,6-Lutidine:collidine:water = 1:1:1. Add 1-2% diethylamine.
47) 2,4-Lutidine (buffered), saturated with pH 6.2 buffer, 0.022 *M* (8 ml. of *M* $KHPO_4$ + 2 ml. of *M* Na_2HPO_4).
48) 2,4-Lutidine:collidine:water = 1:1:2.
49) 2,6-Lutidine:absolute ethyl alcohol:water = 55:20:25 v/v/v + 1-2% diethylamine.
50) 2,4-Lutidine:methyl alcohol:urea:water = 18:80:0.5:2 v/v/w/v.

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[*] Lutidine may be purified by refluxing over ethyl *p*-toluenesulfonate (Cathcart, 1951).

51) Mesityl oxide:formic acid:water = 1:1:2.
52) Methyl alcohol:*n*-butyl alcohol:water:0.2 *M* citrate buffer (pH 5.0) = 30:55:15:1; for methionine.
53) Methyl alcohol:water:dimethylamine = 80:20:0.1 v/v/v.
54) Methyl alcohol:ethyl alcohol:water:urea = 45:45:10:0.5 v/v/v/w.
55) Methyl alcohol:pyridine:water = 80:4:20.
56) *n*-Pentyl alcohol:acetic acid:water = 20:3:10; for thyroxine, etc.
57) Isopentyl alcohol, saturated at 20° C. with 6 *N* ammonia; for diiodotyrosine, etc.
58) Phenol[*]:water = 100:20 v/v Mallinckrodt "Special for Chromatography" liquid phenol. Use in the presence of HCN (NaCN + H_2O), 0.3% NH_3, or 1:1 acetic acid.
59) Phenol, saturated with water, with added 0.002% 8-hydroxyquinoline.
60) Phenol:water:formic acid = 79:20:1 in an atmosphere of NH_4OH + HCN + coal gas.
61) Phenol (buffered). Saturate with pH 12.0 phosphate buffer (50 ml. of 0.067 *M* NaH_2PO_4 + 50 ml. of 0.067 *M* NaOH). Treat the paper with the same buffer before chromatography.
62) Phenol (buffered) = 100:20 buffer w/v. Buffer = 6.3% sodium citrate, 3.7% KH_2PO_4, and 0.5% ascorbic acid.
63) Phenol:*m*-cresol:pH 9.3 buffer = 25:25:7 w/w/v. Buffer = 200 ml. 0.1 *N* boric acid + 113.5 ml. of 0.1 *N* NaOH. This is used as the second solvent of a two-dimensional chromatogram. The paper, except the portion where the amino acids are, is sprayed with the buffer.
64) Phenol:ethyl alcohol:water = 3:1:1; for pipecolic acid.
65) γ-Picoline:water = 60:40.
66) *n*-Propyl alcohol:acetic acid:water = 75:5:20.
67) Pyridine:acetic acid:water = 50:35:15.
68) Pyridine:amyl alcohol:water = 35:35:30 v/v/v.
69) Pyridine:isoamyl alcohol:water:diethylamine = 10:10:7:0.3.
70) Pyridine:water = 65:35.

The type of distribution of amino acids on one- and two-dimensional chromatograms using various solvents are shown in Figures 8 to 36 inclusive.

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[*] Phenol may be purified by extracting 50 g. with petroleum ether (b.p 40-60° C.) at 50-55° C. with shaking. If only one phase results, add 1-2 ml. of water. Remove the aqueous layer. Crystallize from petroleum ether (Mars, 1953).

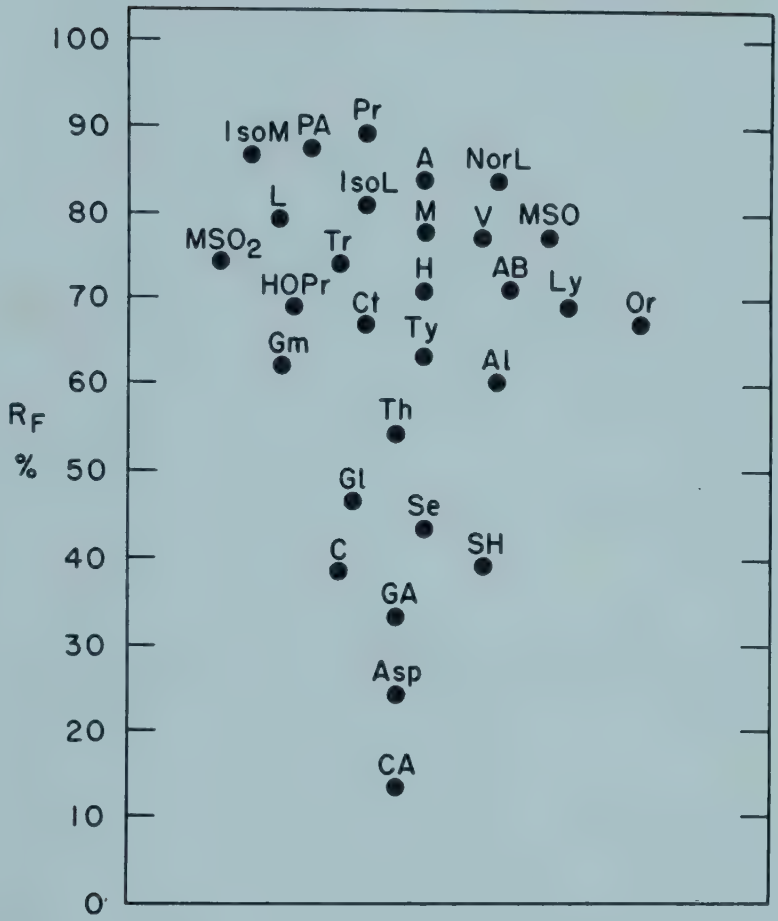

FIGURE 8. Water-saturated Phenol + HCN + 0.3% NH_3

Abbreviations for Figures 8-35, inclusive

A	Arginine	L	Leucine
H	Histidine	IsoL	Isoleucine
Ly	Lysine	NorL	Norleucine
Or	Ornithine	V	Valine
Ct	Citrulline	GA	Glutamic Acid
Gm	Glutamine	Asp	Aspartic Acid
Ty	Tyrosine	AD	a-Amino Adipic Acid
PA	Phenylalanine	Gl	Glycine
C	Cystine	Al	Alaine
SH	Cysteine	AB	α-Amino Butyric Acid
M	Methionine	Pr	Proline
MSO	Methionine Sulfoxide	HOPr	Hydroxyproline
MSO_2	Methionine Sulfone	Tr	Tryptophan
Th	Threonine	IsoM	Isomethionine
Se	Serine	CA	Cysteic Acid

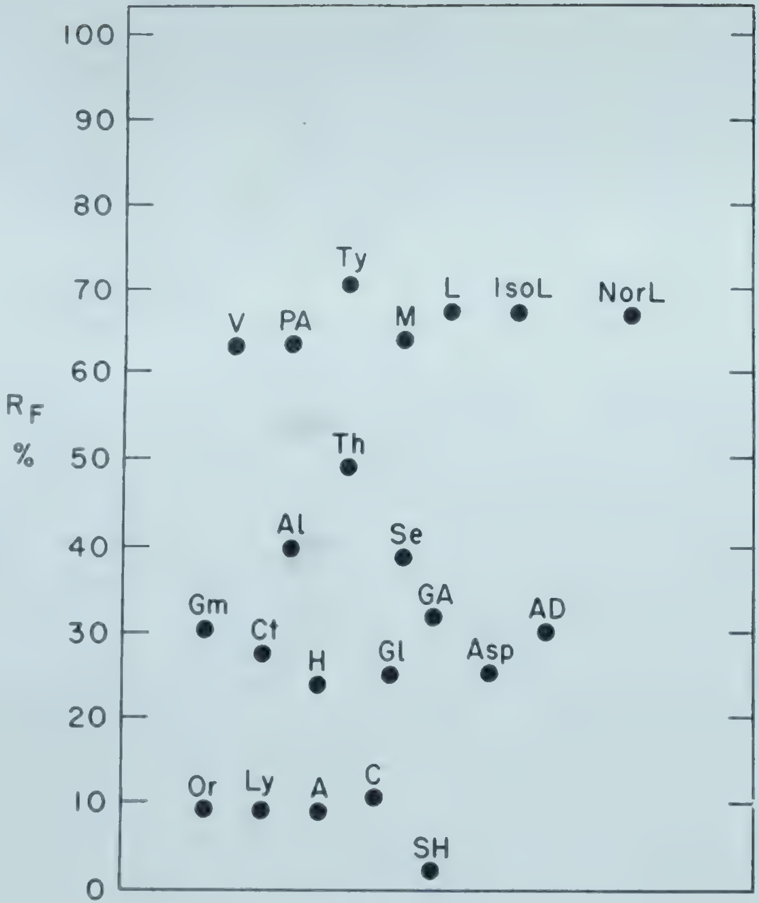

FIGURE 9. 80% Pyridine

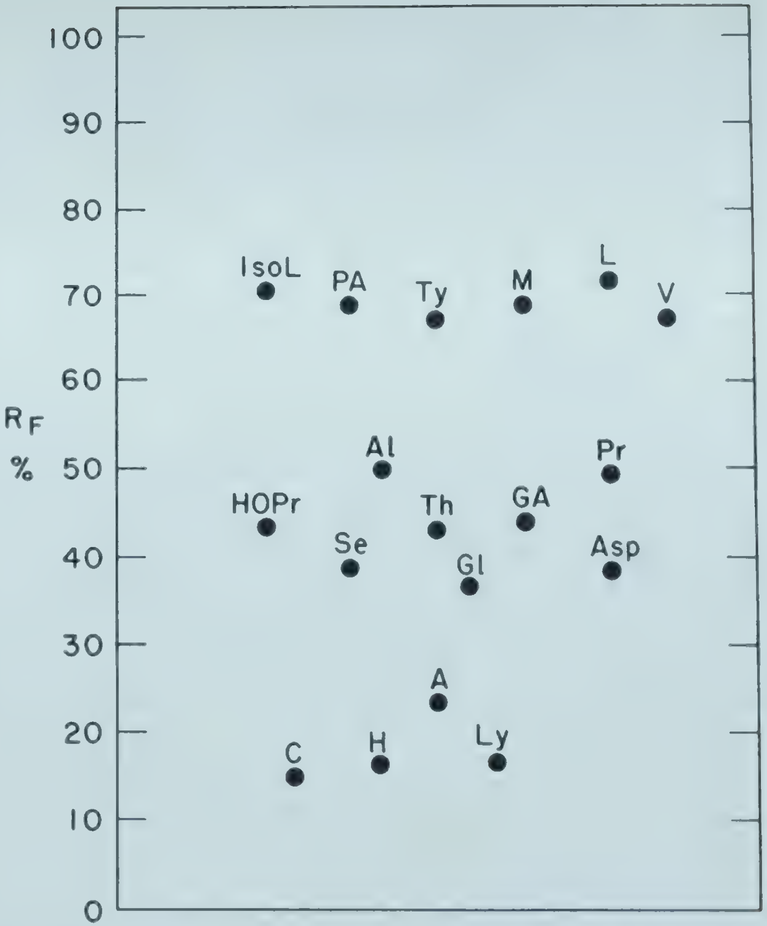

FIGURE 10. 1-Butanol:1-Propanol:0.1 N HCl = 1:1:1 v/v

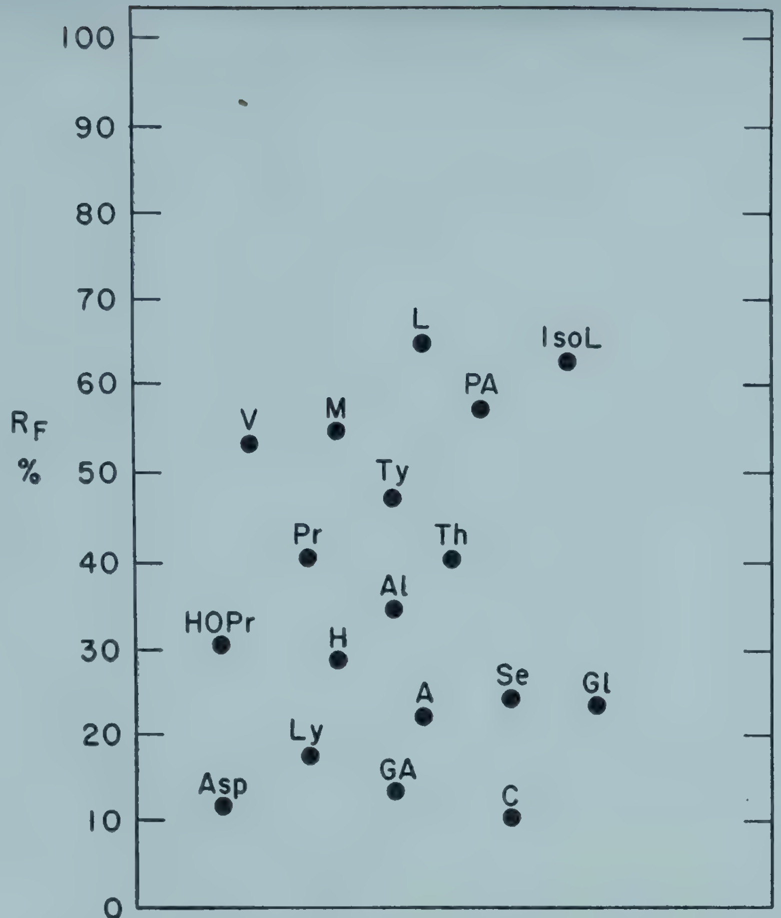

FIGURE 11. 1-Butanol: 1-Propanol:0.1 *N* HCl = 1:2:1 v/v + 0.3% NH_3

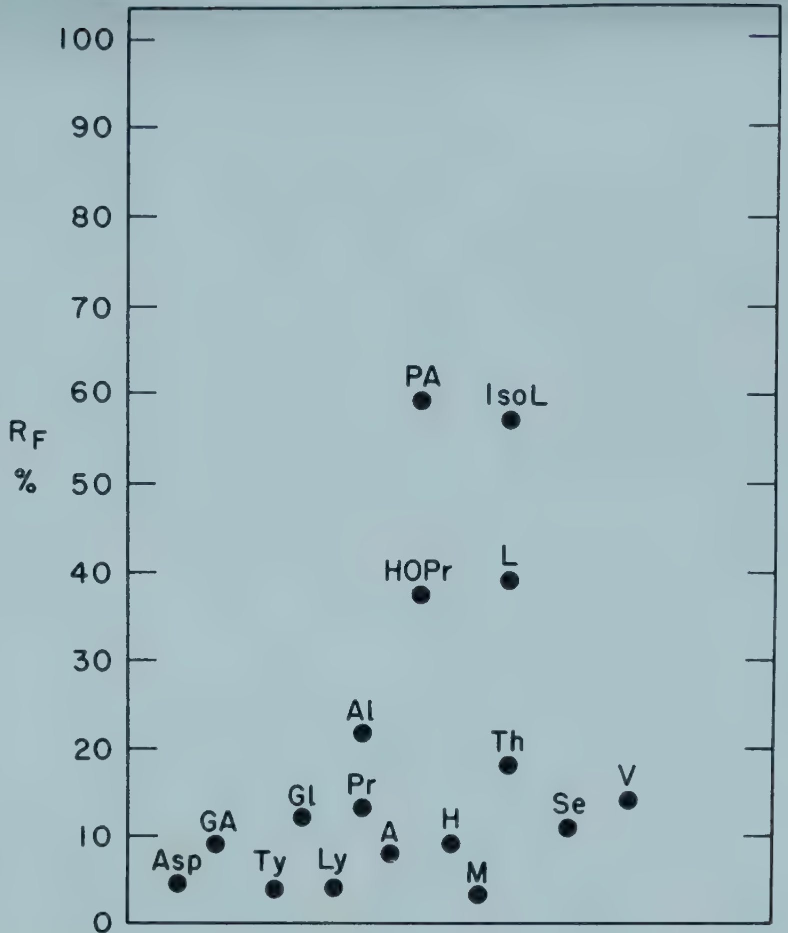

FIGURE 12. Isobutyric acid:Isovaleric acid = 1:1 $^{v}/v$, water-saturated + 0.4% NH_3

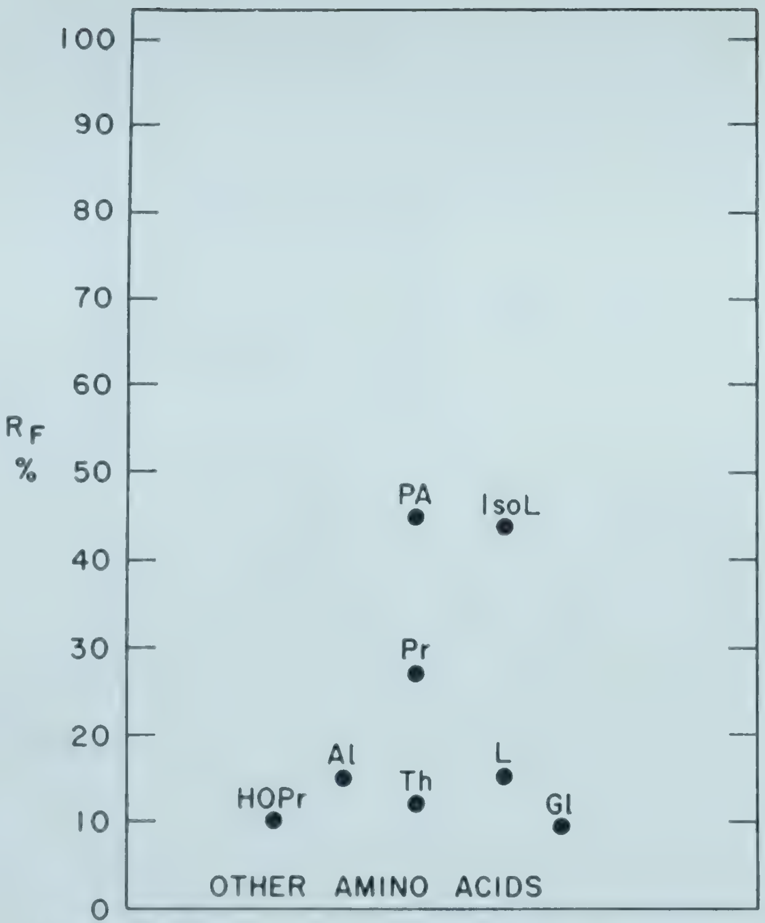

FIGURE 13. Isobutyric acid:Isovaleric acid = 1:1 v/v, water-saturated

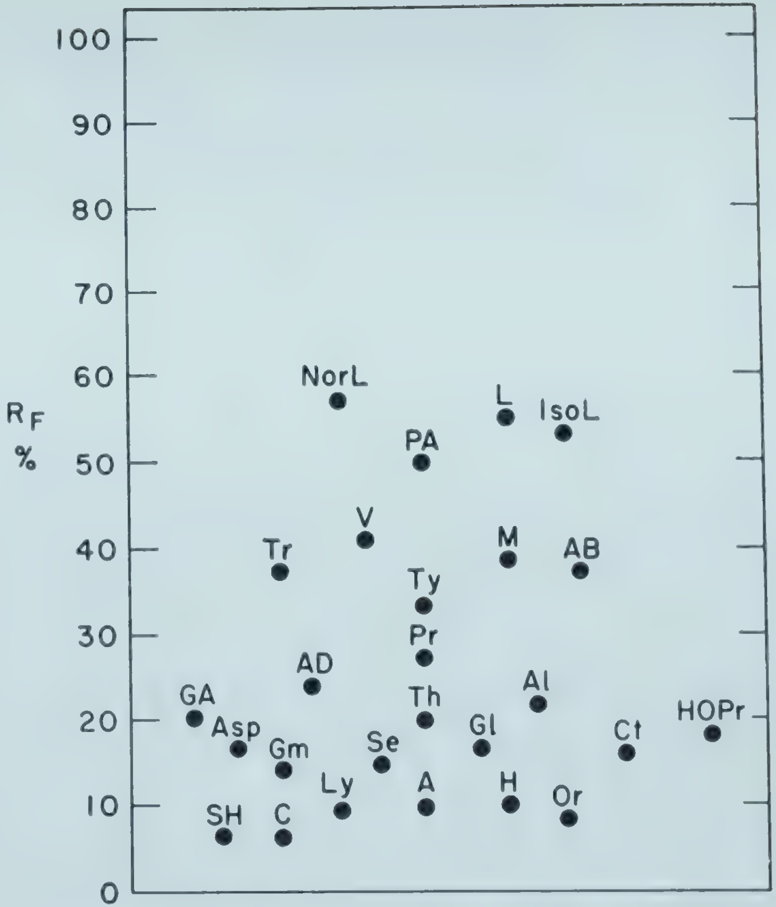

FIGURE 14. 1-Butanol:glacial Acetic acid = 10:1 v/v, water-saturated

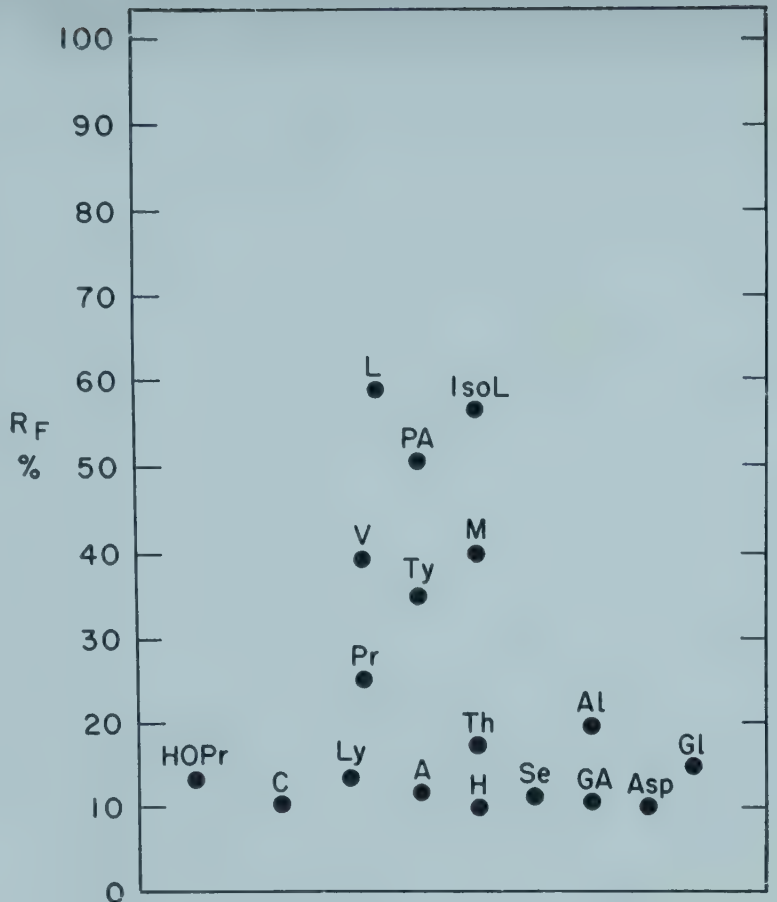

FIGURE 15. 1-Butanol:glacial Acetic acid = 10:1 v/v, water-saturated + 0.3% NH_3

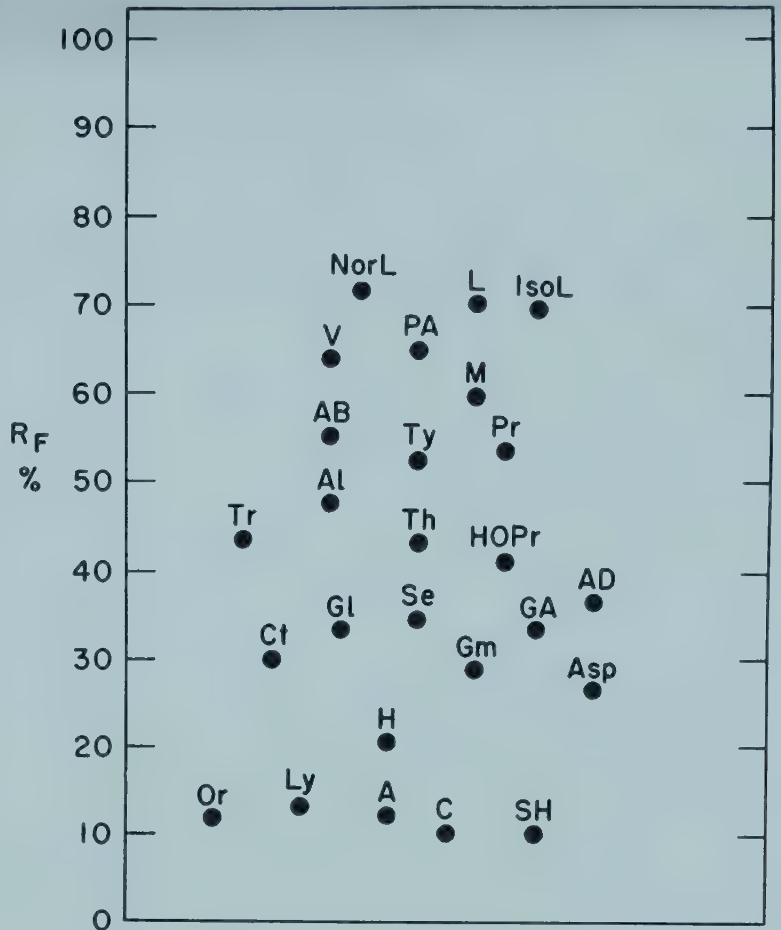

FIGURE 16. 77% Ethanol

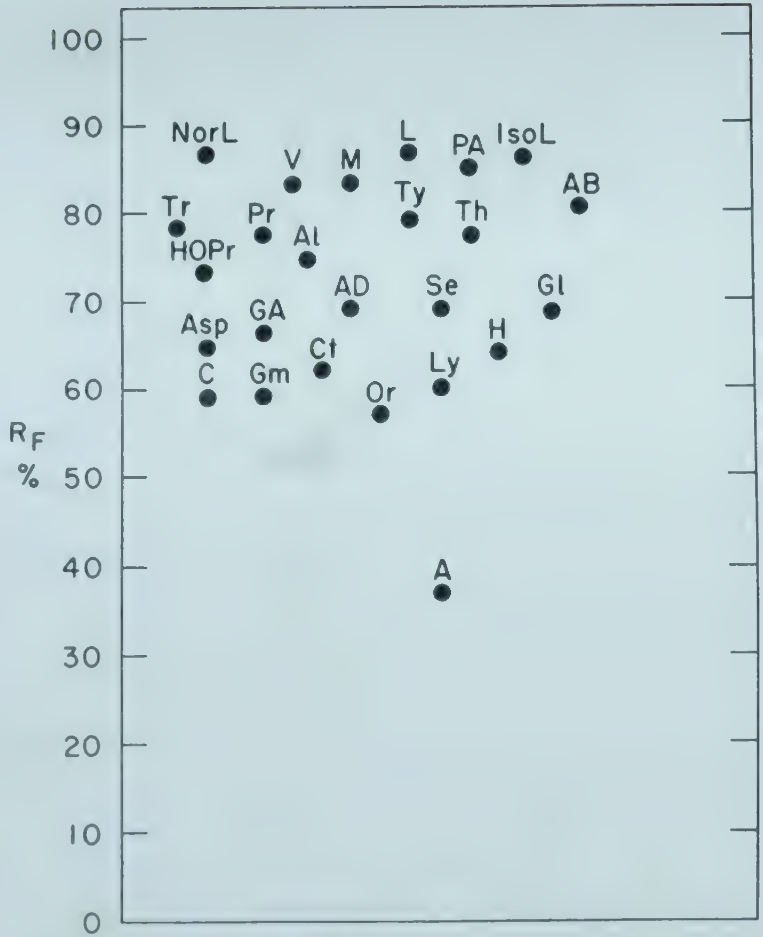

FIGURE 17. 77% Ethanol + Diethylamine

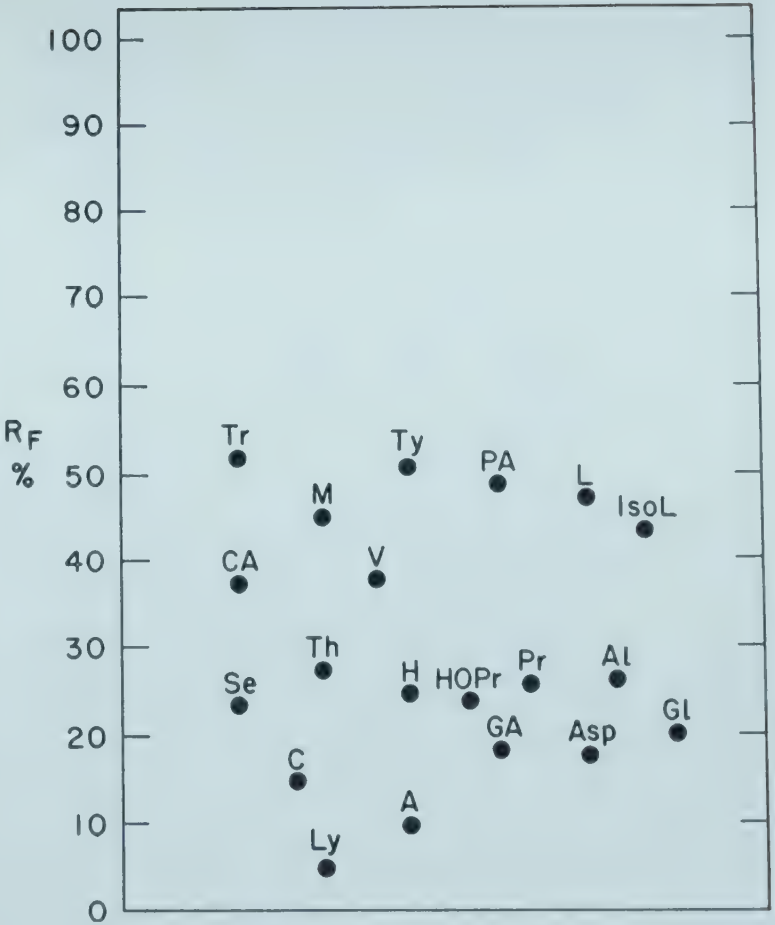

FIGURE 18. 2,4,6-Collidine, water-saturated

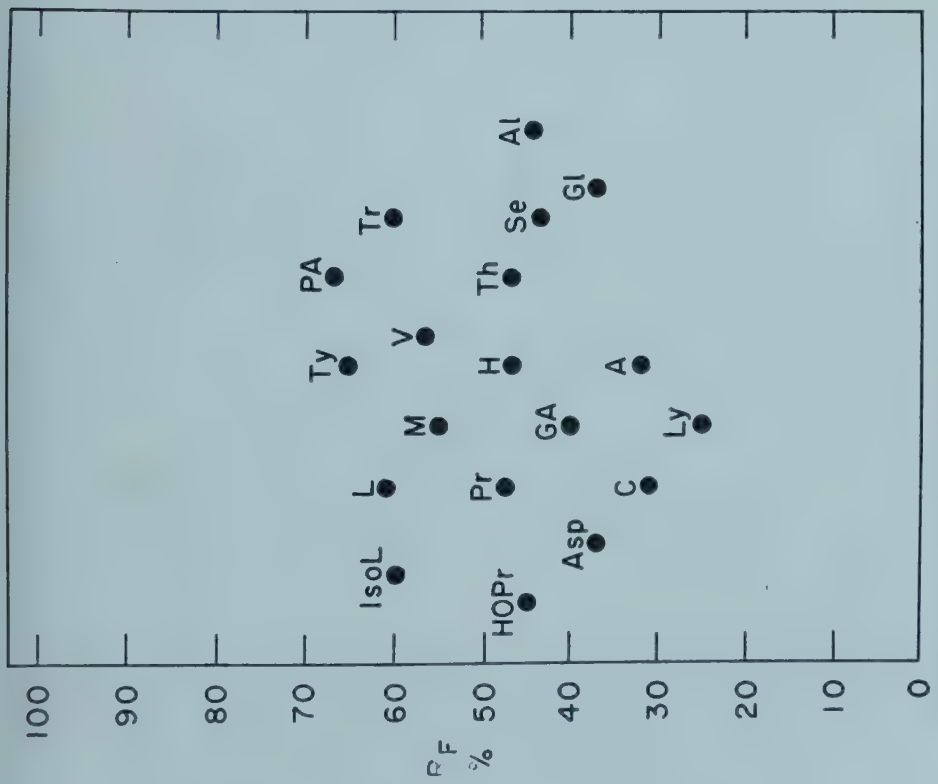

FIGURE 19. 2,4,6-Collidine:2,6-Lutidine = 1:1 v/v, water-saturated

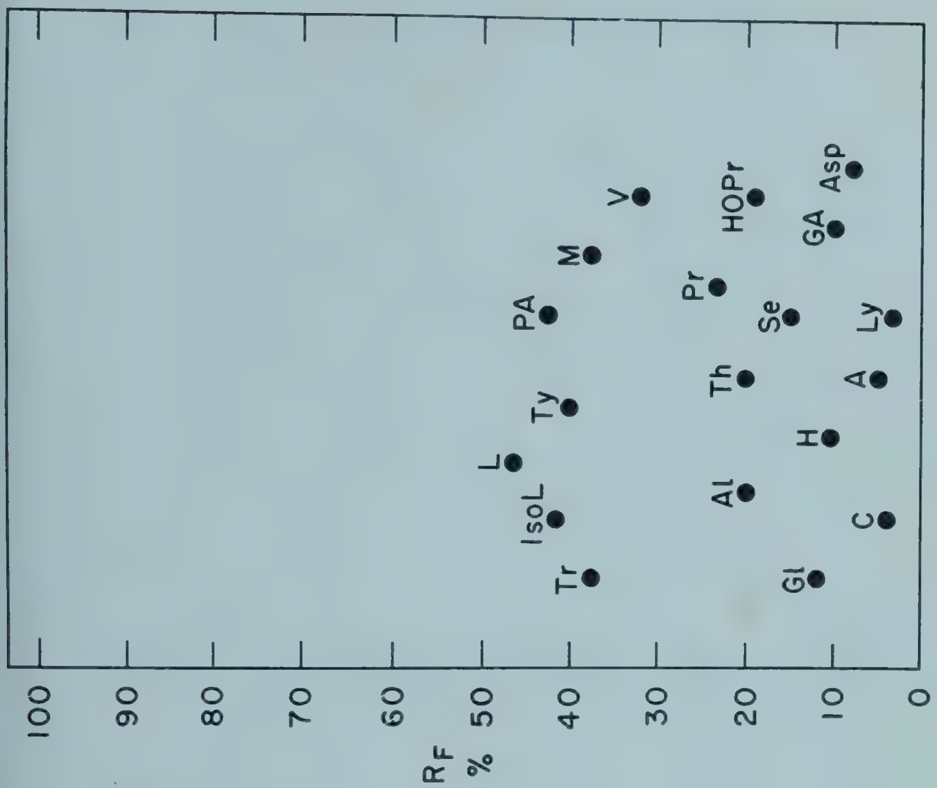

FIGURE 20. 2,6-Lutidine:Ethanol:Water = 3:1:1

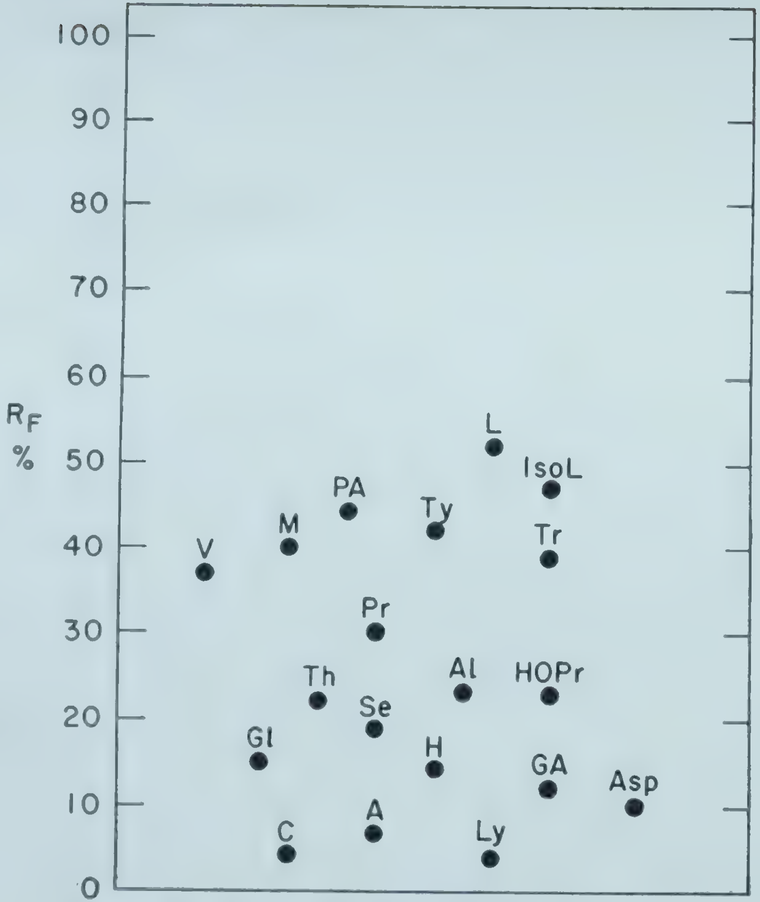

FIGURE 21. 2,6-Lutidine:Ethanol:Water = 5:3:2

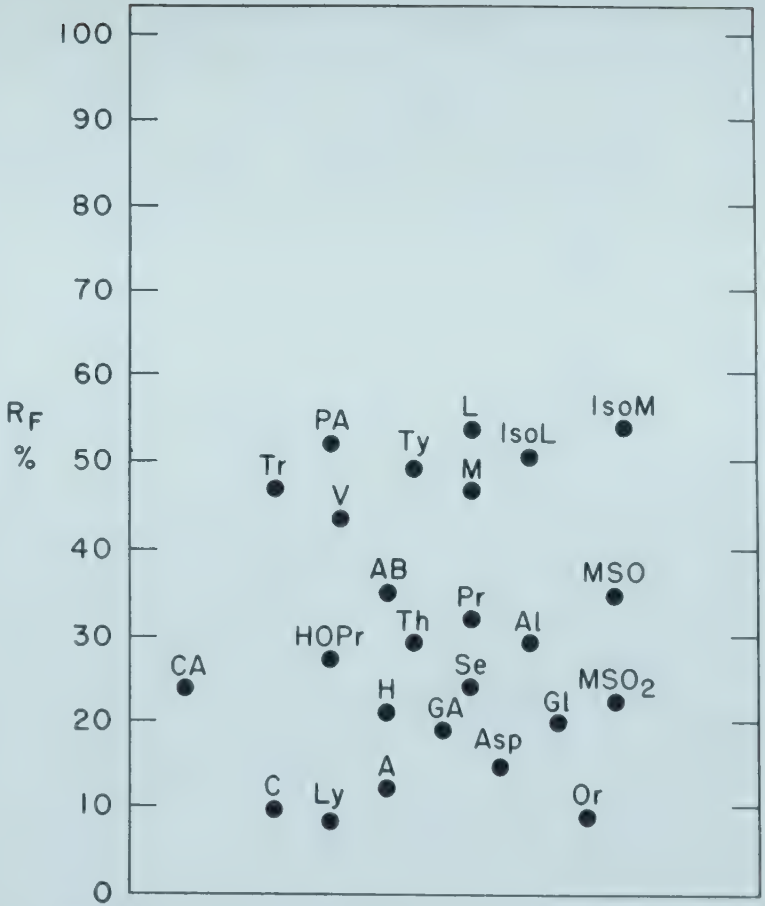

FIGURE 22. 2,6-Lutidine:Ethanol:Water = 55:20:25

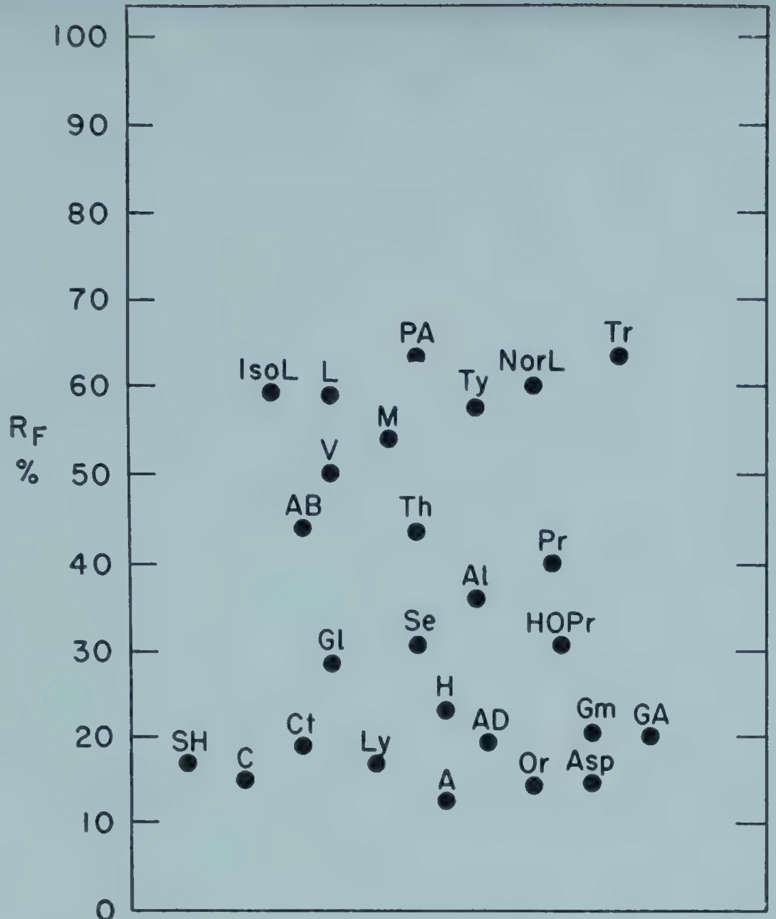

FIGURE 23. 2,6-Lutidine:Ethanol:Water = 55:20:25 v/v + Diethylamine

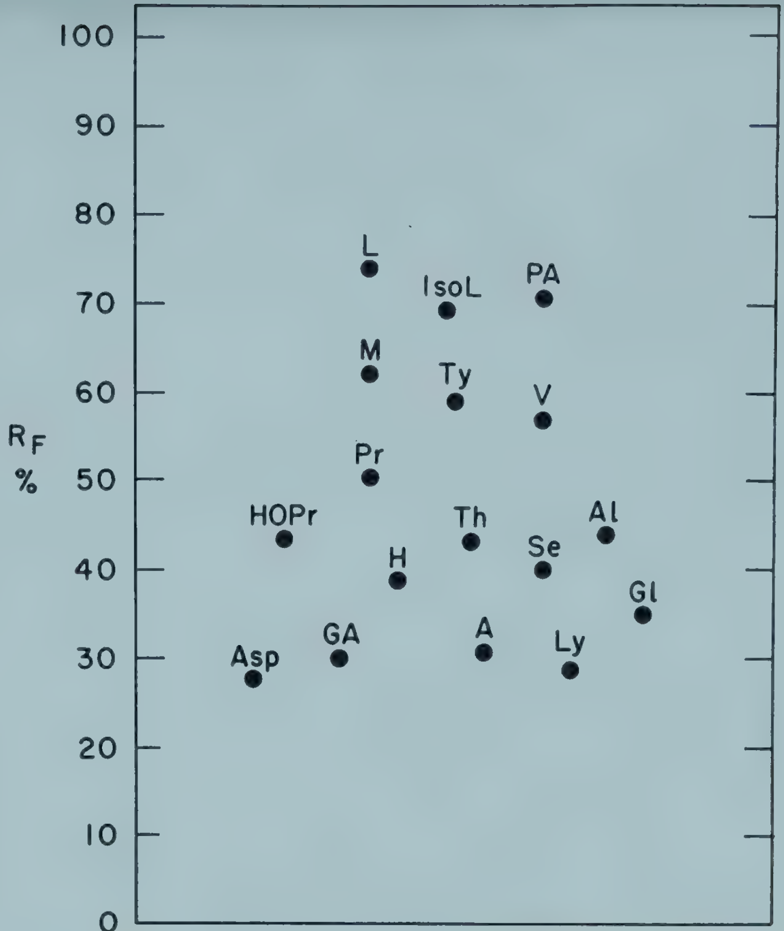

FIGURE 24. 2-Butanol:3-Butanol:Water = 4:1:3 + 6 *N* HCl

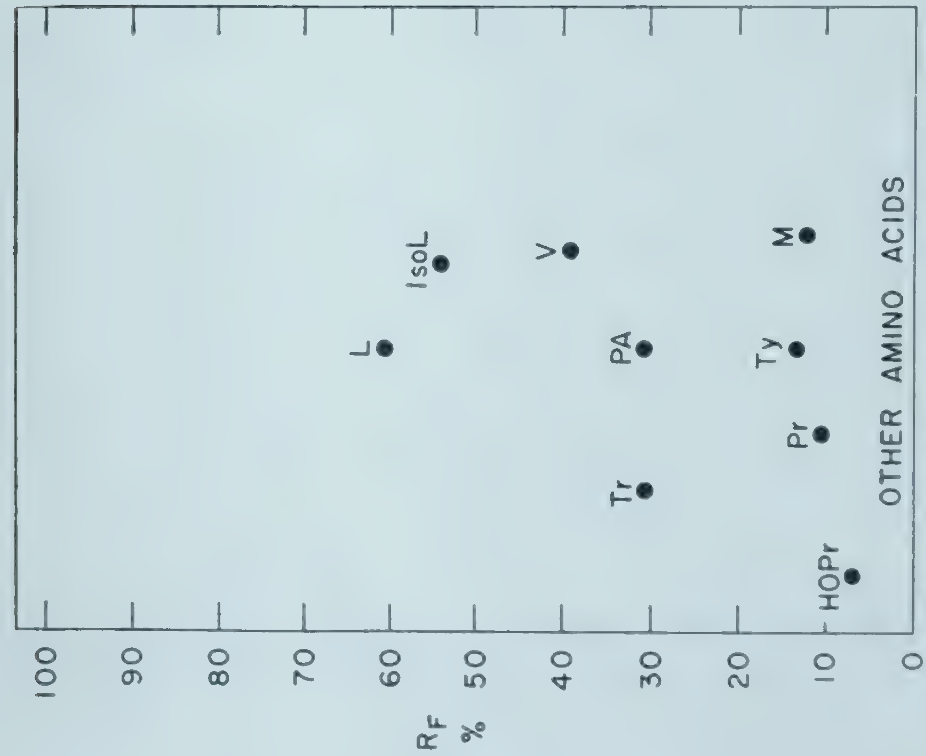

FIGURE 25. 1-Butanol:Benzyl alcohol — 1:1 v/v, water-saturated + diethylamine

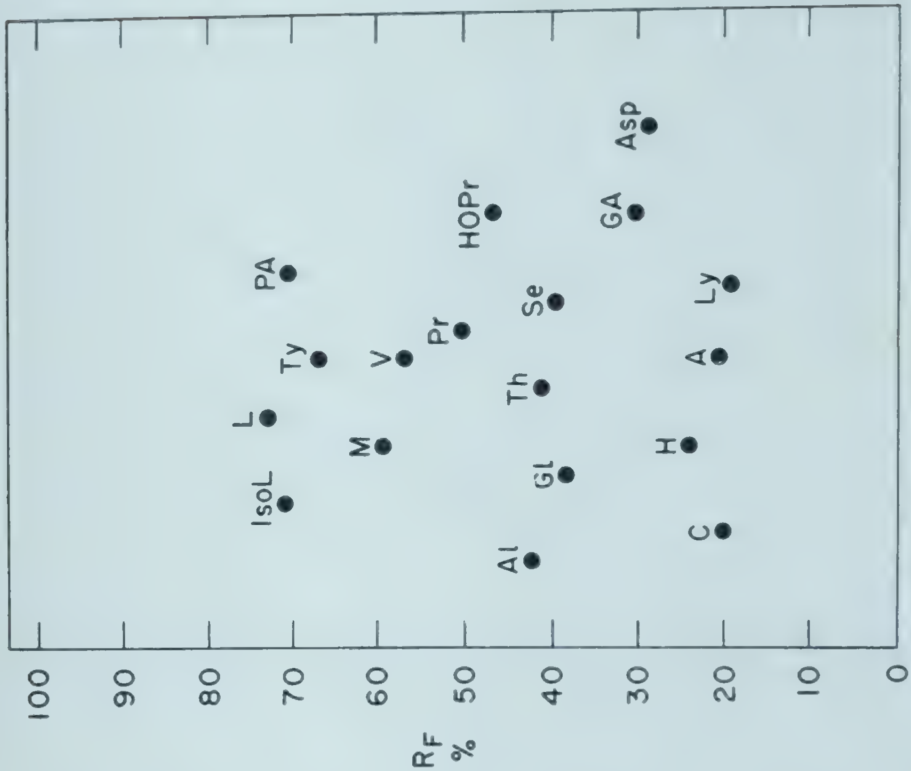

FIGURE 26. 2-Butanol:3-Butanol:Water — 4:1:3

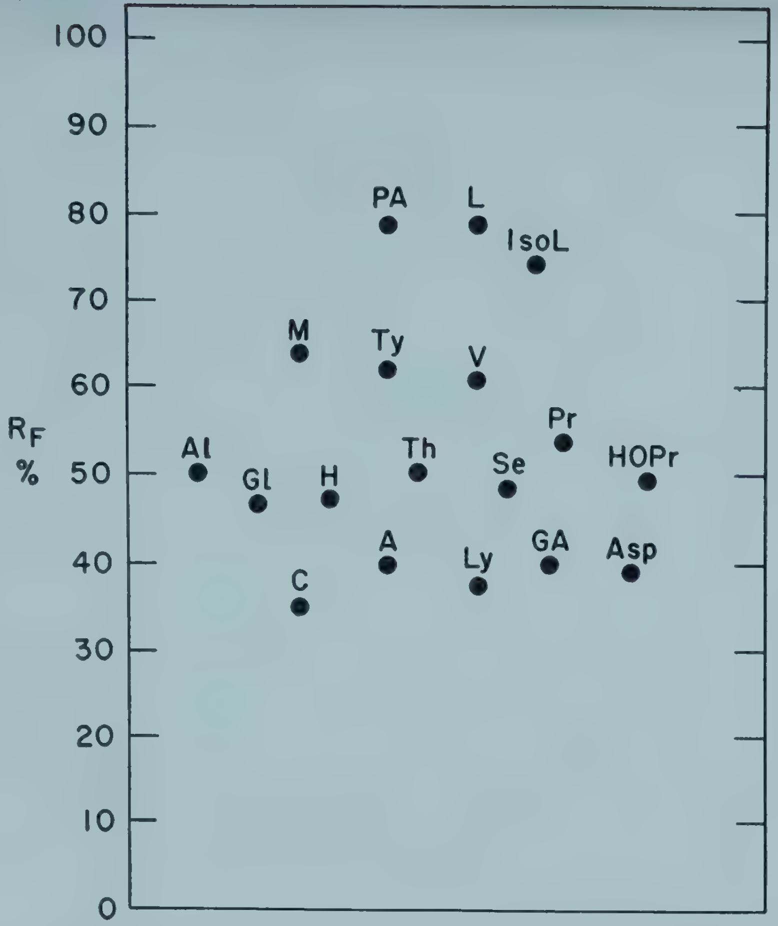

FIGURE 27. 2-Butanol:3-Butanol:Water = 4:1:3 + 0.3% NH_3

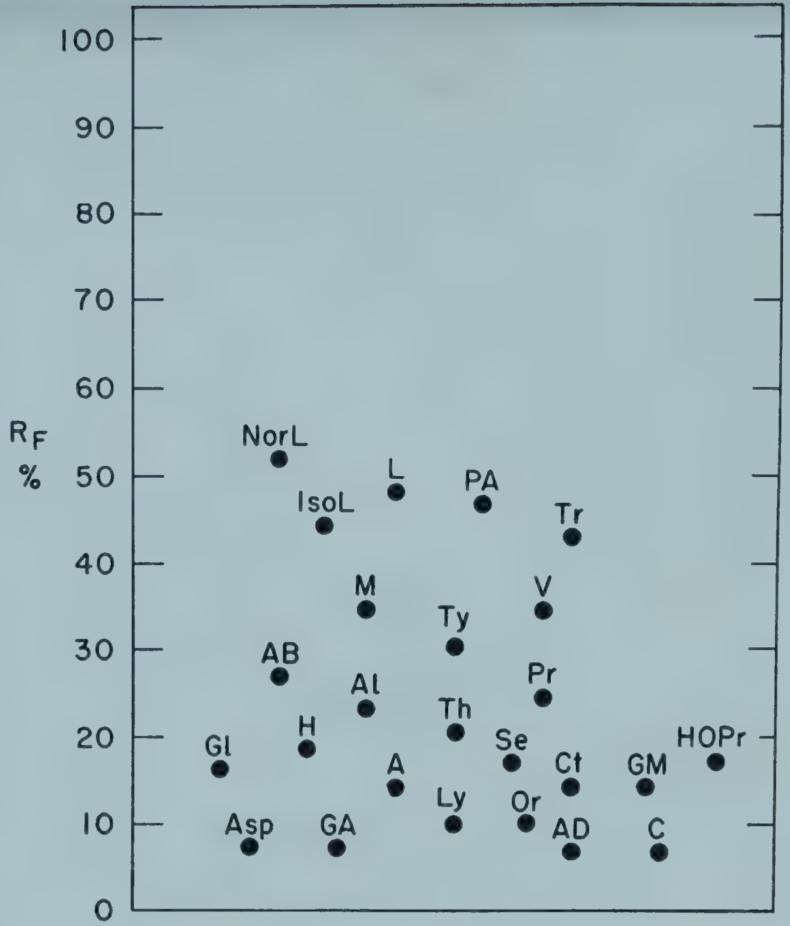

FIGURE 28. 2-Butanol:3% NH_3 = 3:1

Two-dimensional Paper Chromatograms

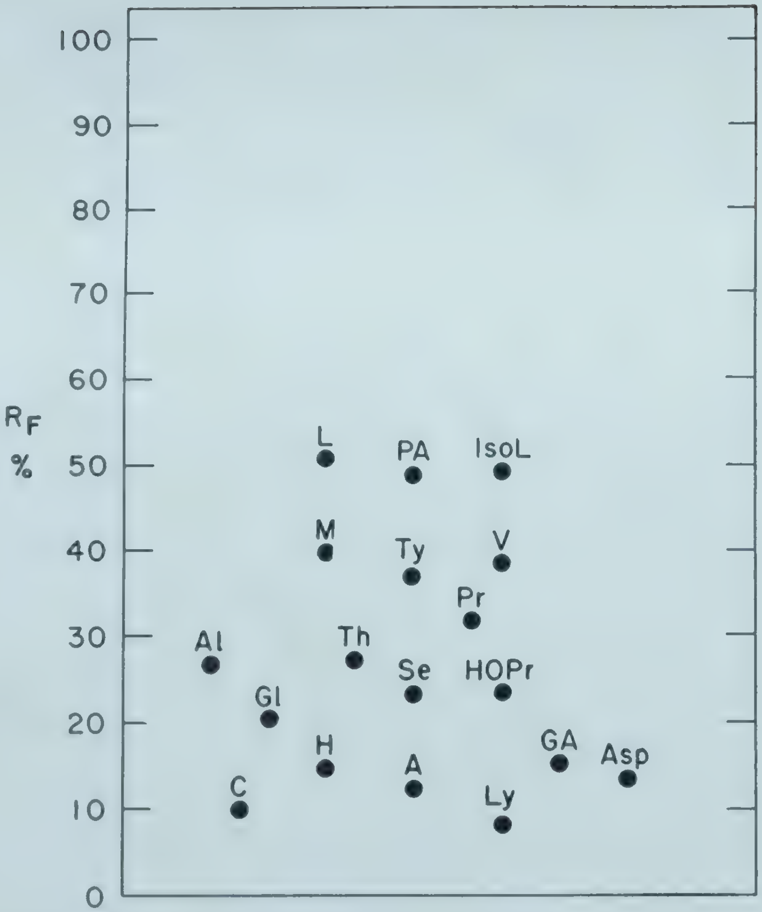

FIGURE 29. 1-Butanol:Dioxane = 4:1 v/v, water-saturated

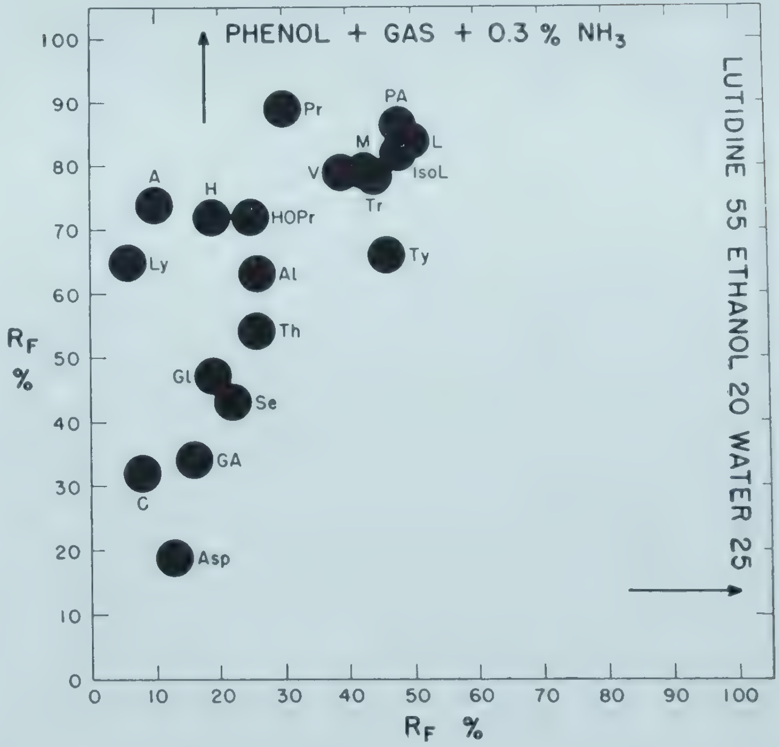

FIGURE 30.

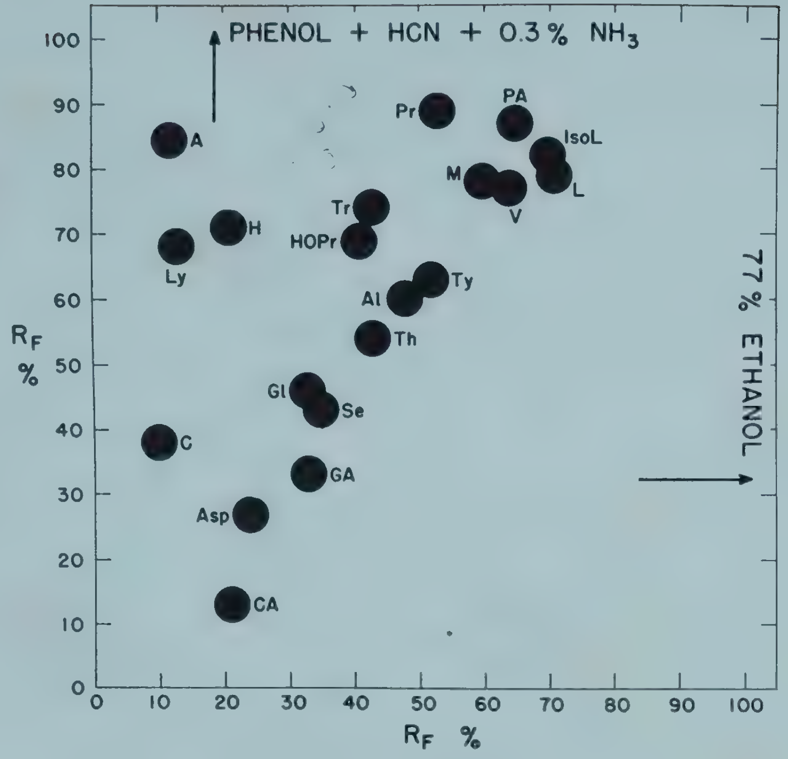

FIGURE 31.

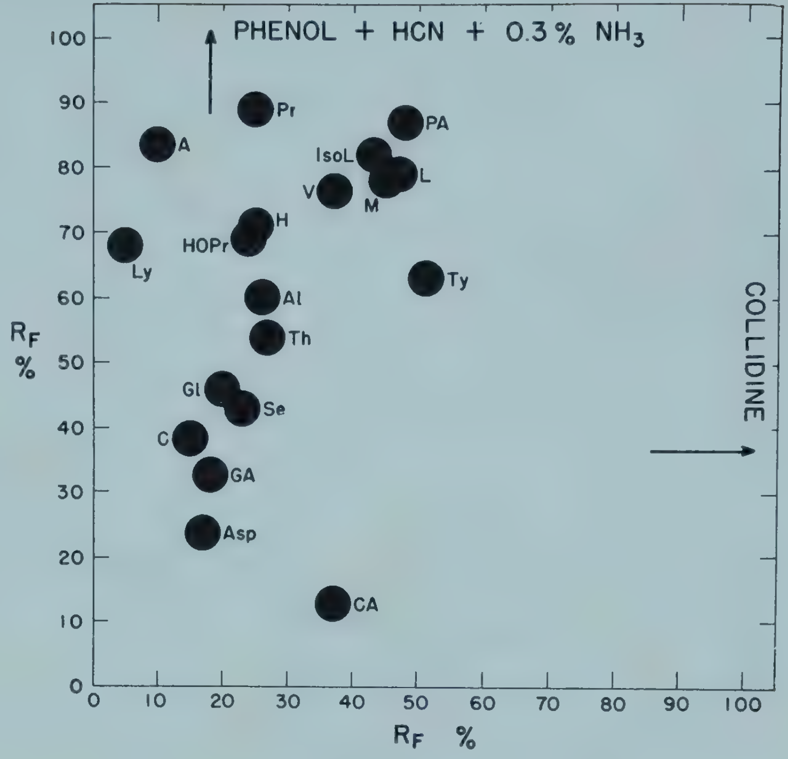

FIGURE 32.

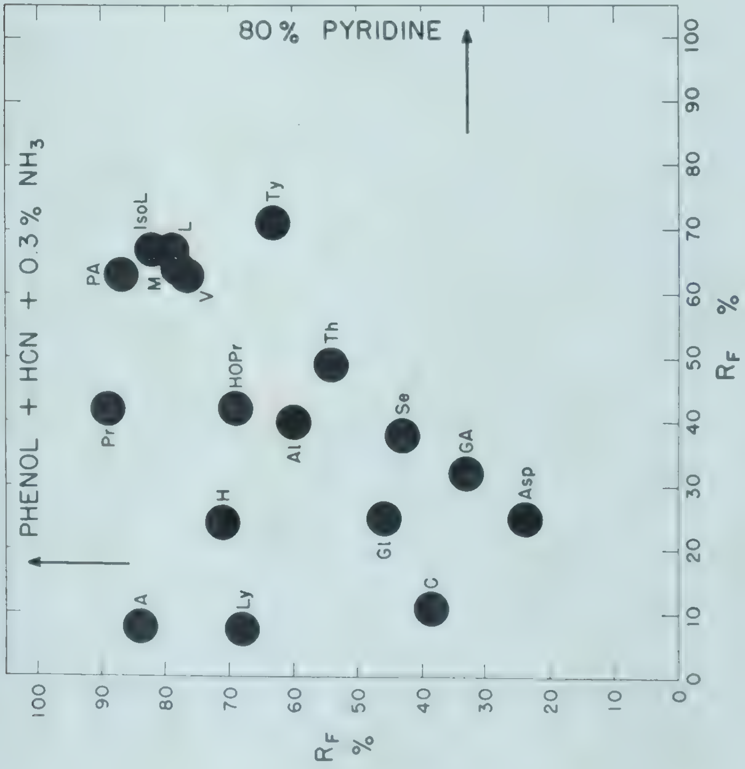

FIGURE 33.

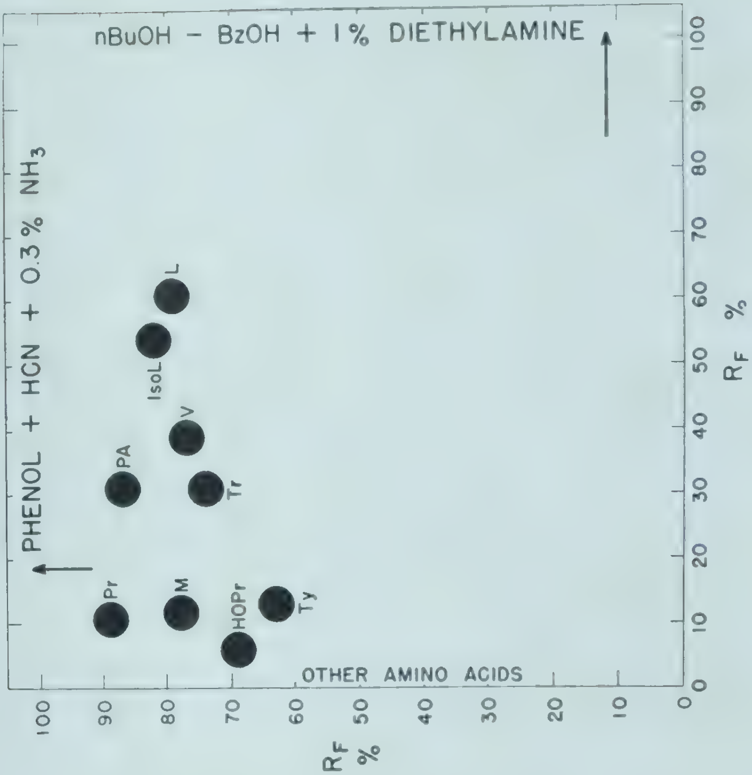

FIGURE 34.

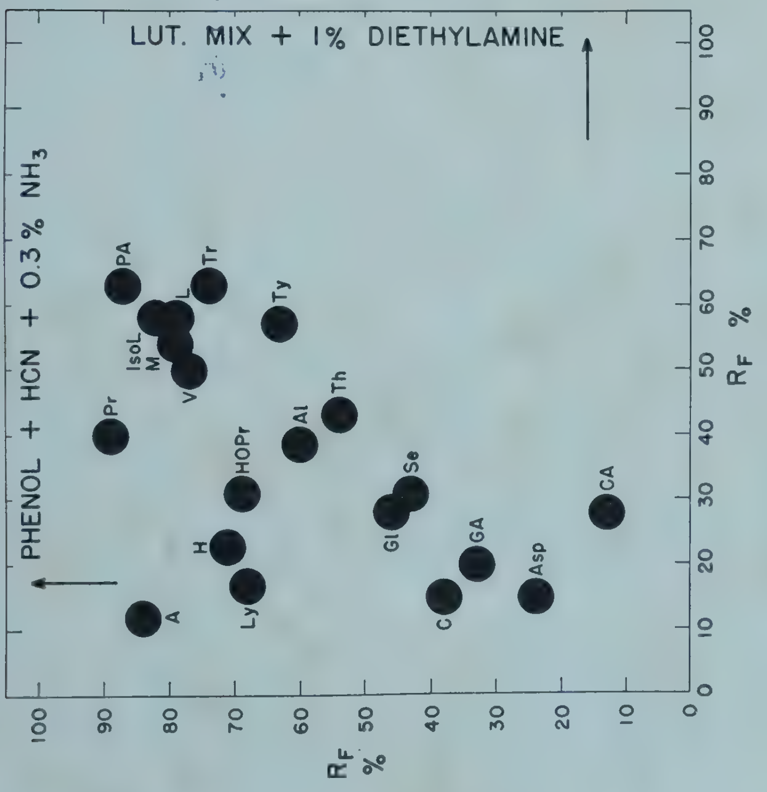

FIGURE 35.

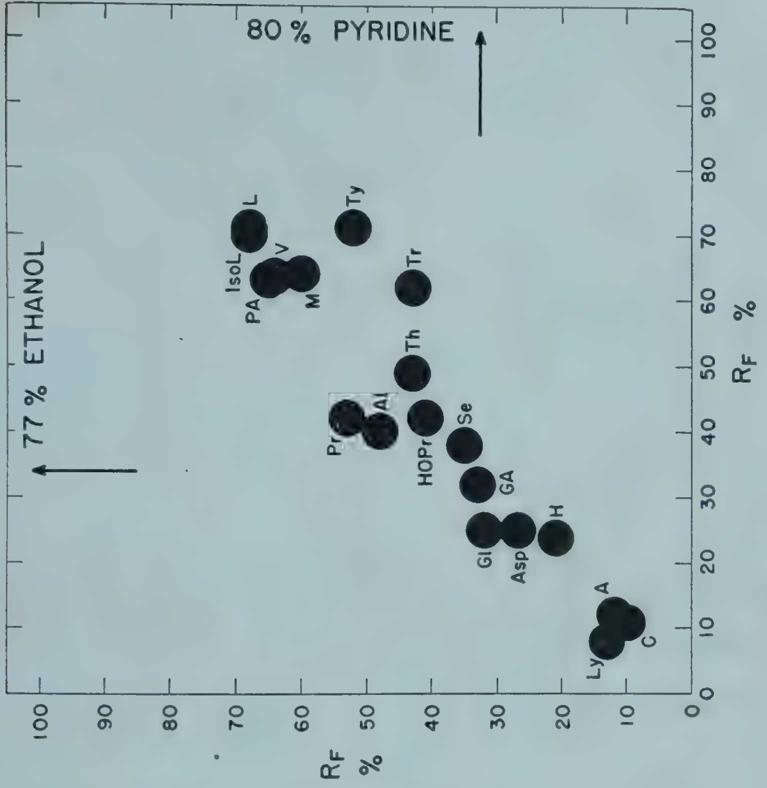

FIGURE 36.

DETERMINATION OF AMINO ACIDS BY COLORIMETRY AFTER ELUTION OF THE SPOTS ON TWO-DIMENSIONAL CHROMATOGRAMS

The procedure given below is that developed by Levy (1954b, 1955ab).

1. Dinitrophenylation

Dinitrophenylation is effected quantitatively by stirring an aqueous solution of the amino acids (20-30 μM. in 3 ml.) with a slight excess of 1-fluoro-2,4-dinitrobenzene (FDNB) for 80 minutes at pH 9.0 and 40° C., the pH being maintained at this value throughout this period by intermittent additions of standard alkali (2 *N* NaOH or Na_2CO_3). Excess FDNB is then extracted with ether, the solution is acidified, and the amino acids are extracted into ether (5 × 5 ml.). The aqueous solution, which contains DNP-arginine and α-DNP-histidine, is diluted to 10 ml. (*cf.*, Sanger, 1945, 1951; Green, F. C., 1952).

2. Chromatography

Paper: Whatman No. 1.

Solvents: Toluene:chloroethanol:pyridine:0.8 *N* ammonia = 10:6:3:6 v/v. Phosphate buffer, 1.5 *M.* *M* NaH_2PO_4 + 0.5 *M* Na_2HPO_4.

Method: A 2-ml. aliquot of the ether solution and a 1.0-ml. aliquot of the water solution are applied to adjacent corners of an 18¼″ x 22½″ sheet of Whatman No. 1 filter paper, which is then irrigated by the ascending procedure with the toluene:chloroethanol:pyridine:ammonia solvent. The chromatogram is dried for 3 to 4 hours at 40° C., and the spots due to DNP-arginine and α-DNP-histidine are excised at this point. The paper is then run in the second dimension by the descending procedure with aqueous phosphate buffer. All the ether-soluble DNP-amino acids are thereby separated with the exception of DNP-leucine and DNP-isoleucine. The positions of DNP-tryptophan and di-DNP-histidine coincide, but this does not present a difficulty in practice, since the former amino acid is not normally present in acid hydrolyzates and the latter amino acid is determined as its mono-DNP-derivative.

The spots are cut out and dropped into a set of labeled test tubes; three blanks are also cut from each sheet (see Figure 37). Four ml. of water are pipetted into each of the tubes, which are then placed in a water bath at 55-60° C. for 15 minutes to allow complete elution of the color. After an additional 15 minutes to allow the solutions to cool to room temperature, they are successively decanted into a 1-cm.

quartz cuvette, and the optical densities at 360 mμ (385 mμ in the case of DNP-proline) are read in the Beckman Model DU spectrophotometer against a water blank. The optical density reading of the tubes containing blank paper is 0.001-0.002 per square centimeter, and the appropriate corrections are made for each spot according to its estimated size.

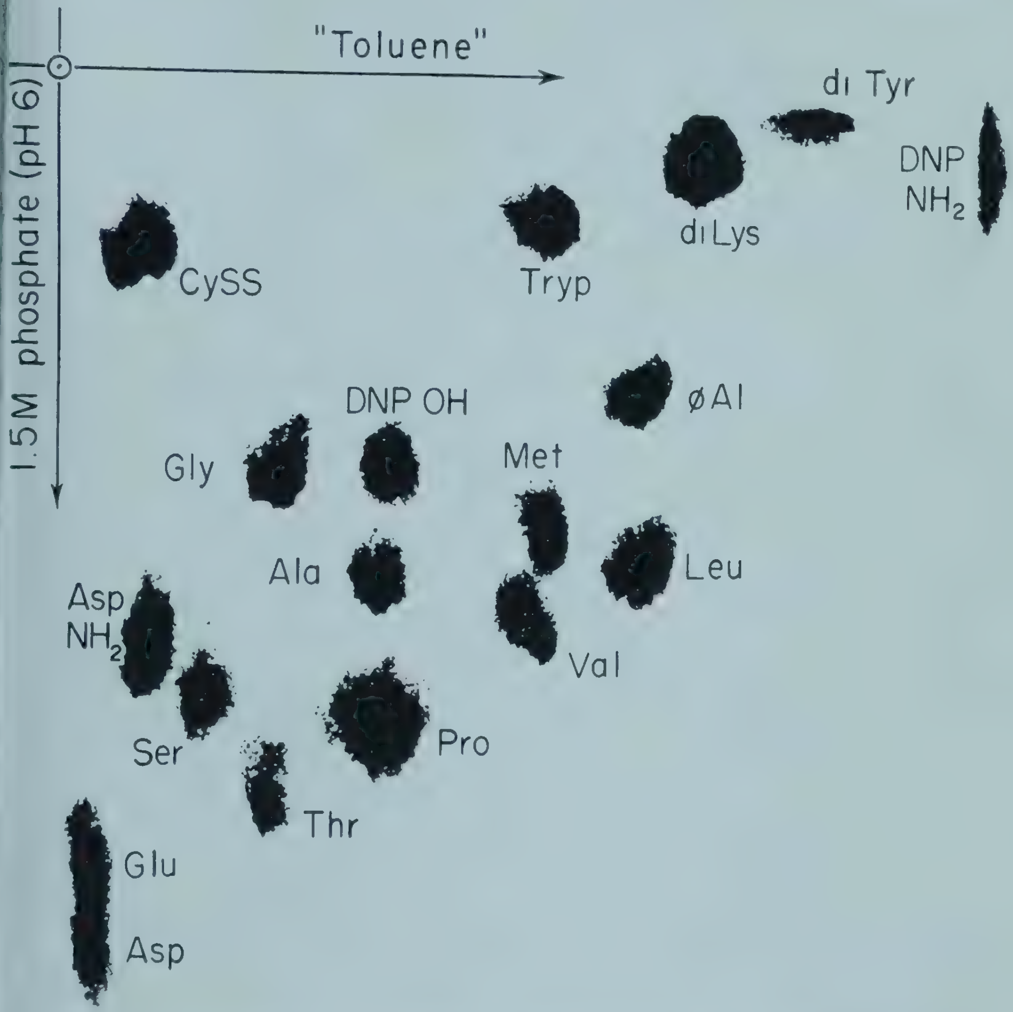

FIGURE 37. Two-dimensional chromatogram of a synthetic mixture of DNP-amino acids (approximately 0.02 μM of each). (After Levy, 1954.)

3. Calculations

The resulting optical density ratios of the DNP-amino acids are converted to molar ratios by multiplying by the following factors, which, within the accuracy of the method (± 4%), are independent of the composition of the mixture analyzed: Asp, 0.99; Glu, 0.94;

CySS, 0.56; Ser, 0.97; Thr, 1.02; Gly, 1.03; Ala, 1.09; Pro, 0.93; Val, 0.99; Met, 1.21; Leu and Isoleu, 1.10; Phe, 10.3; Try, 1.54; Lys, 0.64; His, 1.62; Arg, 1.06. When the chromatograms are run in triplicate, the molar ratios are found to be reproducible to 0-10%.

When it is necessary to estimate the absolute amounts of the amino acids present in the mixture, two independent methods of calculation are available. The less accurate one (2-5%) involves a direct conversion of the optical density readings to micromoles by means of a set of effective millimolar coefficients; they have the values of 15.6 Fm where F represents the above set of factors. The greater inaccuracy of this procedure arises from differences in the absolute recovery of color from replicate chromatogams, these differences are not reflected in the molar ratios, however, since the variation seems to affect each amino acid to a comparable extent. The preferable method, therefore, combines the more accurate (2-3%) molar ratios with the total quantity of amino acids present in the mixture; the latter figure, in turn, can readily be deduced from the alkali uptake which accompanies the reaction of the amino acid mixture with FDNB. Thus, under the above-mentioned reaction conditions, the alkali uptake is found to be 77 1% of the theoretical maximum (*i.e.*, 2 equivalents per NH_2 group, 1 equivalent for tyrosine-OH, and 0.25 equivalent for the imidazole ring of histidine), after due correction is made for the formation of dinitrophenol (0.44 μM of OH ml.$^{-1}$ min.^{1}). The latter by-product does not interfere with the chromatography when the condensation with FDNB is carried out as described above in a solution approximately 0.01 *M* with respect to amino acids; for more dilute solutions, however, it is necessary to reduce the dinitrophenol concentration by sublimation (Mills, 1952) before application to the paper.

4. Comments

Heikens (1954) has shown that polymerization reactions may take place during dinitrophenylation. This observation indicates that care must be taken during the preparation of the DNP-derivatives of the amino acids. The preparation, purification and composition of the DNP-amino acids has been given by Rao (1954). The DNP-amino acids may also be separated on a silica-gel-celite column (Green, F. C., 1952). Zeile (1949) used the p-phenylazoanilinoformyl amino acids in place of the DNP-derivatives (*cf.*, Turba, 1948b; Schlögl, 1954).

CHAPTER VI

The Determination of Amino Acids by Column Chromatography on Ion Exchange Resins

By **William G. Gordon, Ph.D.**

INTRODUCTION

IN 1951 MOORE and Stein described a procedure for the chromatographic separation of mixtures of amino acids by elution analysis on columns of Dowex 50, a synthetic ion-exchange resin. In conjunction with a photometric ninhydrin method previously reported by these authors, whereby quantitative analysis of the effluent fractions may be carried out, the procedure permits accurate determination of the commonly occurring amino acids in acid hydrolyzates of proteins. The reliability of these techniques has now been amply demonstrated through successful use in many different laboratories. It is quite clear that these contributions to precise amino acid analysis are of great importance.

In this chapter primary emphasis will be placed on the experimental details of the ion-exchange method and its use in amino acid analysis of proteins. Only a few pertinent articles will be cited as illustrative of the earlier developments in column chromatography of amino acids. Reviews of the subject by Wieland (1943), Martin (1945), Tiselius (1947), Block (1949c) and Samuelson (1953) may be consulted for additional information.

SEPARATION OF BASIC AMINO ACIDS

Early attempts to apply ion-exchange reactions to the separation of mixtures of amino acids showed that group fractionations, depending primarily on the net charge and sign of charge of the amino acids, were feasible. For example, the experiments of Whitehorn (1923) demonstrated that only the basic amino acids, arginine, lysine and histidine, were adsorbed from neutral solution by Permutit (a synthetic sodium aluminum silicate).

Not only were group separations of basic amino acids possible by these methods, but also, as demonstrated by Turba (1941), separations of the individual diamino acids. Turba used Filtrol, Neutrol and Floridin XXF, ion-exchange materials of the fuller's earth type, in his studies. When a mixture of monoamino and diamino acids was put on a column of Floridin XXF and eluted with water, arginine and lysine remained in the column, while histidine and monoamino acids emerged. Histidine was separated by adsorption on Filtrol-Neutrol and subsequent elution with a sulfuric acid-pyridine mixture. Arginine and lysine were eluted from the original column by sulfuric acid-pyridine; the solvent was removed, the mixture taken up in M/6 KH_2PO_4, and the solution run through a column of Filtrol-Neutrol. Arginine was retained while lysine was recovered from the eluate. The arginine was finally eluted with sulfuric acid-pyridine (*cf.*, Block and Bolling, 1951, p. 36 *et seq.*).

Other ion-exchange materials, such as metallic oxides (TiO_2, Al_2O_3, silica gel) and special synthetic resins, have been used also in these separations. The demonstration by Adams and Holmes (1935) that synthetic resins are capable of exchanging ions, was of general importance in that it became possible to produce materials suited particularly to separations of ions of specific kinds. The first applications of synthetic resins to amino acid separations were made by Block (1942) and independently by Freudenberg (1942) in the same year. Block found that the basic amino acids could be separated from other amino acids and, in later work, that large-scale fractionations of protein hydrolyzates could be made in this way (*cf.*, Block, 1949).

SEPARATION OF DICARBOXYLIC AMINO ACIDS

When Al_2O_3 is treated with dilute acid, it acquires a positive charge and it may then be used as an ion-exchange material to adsorb aspartic and glutamic acids from mixtures of amino acids. This observation by Wieland (1942) was developed by him and others into a method for the separation of the dicarboxylic acids from protein hydrolyzates and for the separation of the two acids from each other (*cf.*, Block and Bolling, 1951, Chapter VI). Thus, Darling (1945) used a column of Al_2O_3, previously treated with N HCl and washed with water, to adsorb aspartic and glutamic acids from a neutralized mixture of amino acids. The other amino acids were washed out with water. Glutamic acid was then eluted with dilute acetic acid, aspartic acid being retained on the column. Dilute KOH served to recover the aspartic acid.

The first use of a synthetic ion-exchange resin for the quantitative separation of dicarboxylic acids from a protein hydrolyzate was that of Cannan (1944). A hydrochloric acid hydrolyzate was treated with enough Amberlite IR-4 (a synthetic polyamine-formaldehyde resin in free-base form) to raise the pH to 7. The dicarboxylic acids could be adsorbed quantitatively by a number of such treatments. The batches of resin were washed with water and the aspartic and glutamic acids recovered together by elution with excess HCl.

Amberlite IR-4 was also used by Consden (1948) to adsorb the dicarboxylic amino acids and, further, to separate them effectively. In this method a column of the resin maintained at pH 3 to 4 retained the dicarboxylic acids in a protein hydrolyzate. After the column was washed with water, glutamic and aspartic acids together were eluted with *N* HCl. Excess HCl was removed and the dicarboxylic acids were transferred to a second column of the resin equilibrated at pH 2.5. At this pH glutamic acid was eluted first, followed by aspartic acid.

SEPARATION OF NEUTRAL AMINO ACIDS

The prime importance of charge in the exchange separations of amino acids described in the preceding paragraphs is obvious. However, other phenomena, such as true adsorption and partition, are also operative in ion-exchange column chromatography. Indeed adsorption chromatography and partition chromatography alone have been used for separations of neutral amino acids.

1. True Adsorption

During the course of a broad investigation of adsorption analysis begun in 1941, Tiselius demonstrated that simple mixtures of neutral amino acids could be separated by virtue of different affinities of the components for active carbon. Important new methods of chromatographic analysis were introduced as the work progressed (*cf.*, Tiselius, 1947; Block and Bolling, 1951, p. 400 *et seq.*). Three different methods used by Tiselius, *elution analysis, frontal analysis* and *displacement analysis,* will be described in general terms.

In *elution analysis,* the normal procedure in column chromatography, a small volume of the solution to be analyzed is forced into the top of a column of washed adsorbent. Pure solvent, in large quantities, is then passed through the column. The components of the mixture move down the column in separate zones. Pure solvent emerges first, followed by a solution of pure, least strongly adsorbed component, then pure solvent, another component, etc. To remove most strongly adsorbed components quantitatively, it is often more efficient to change the composition of the solvent during the course of elution.

In *frontal analysis*, the solution to be analyzed is passed continuously through the column. The different components are retarded relative to the solvent in their passage through the adsorbent, each component exhibiting a specific retardation dependent on the extent of adsorption. If the original solution contains *A* and *B*, both components are adsorbed and move through the column as sharp, separate fronts as the adsorbent becomes saturated with respect to each component. If *B* is more strongly adsorbed than *A*, then pure solvent emerges first, followed by a sharp step in concentration as *A* emerges, and finally, by a second step as *B* emerges together with *A*. The composition of the effluent is then identical with that of the original solution.

In *displacement analysis*, as in elution analysis, a small volume of the solution to be analyzed is introduced into the column but instead of pure solvent as developer, a solution of a substance ("developer") more strongly adsorbed than any of the components is used. Actually a frontal analysis of the "developer" occurs behind the components of the original solution and the latter are pushed through the column just ahead of the advancing front of the "developer." The displaced components gradually form sharply separated zones, each containing a single component. Pure solvent emerges first, followed by the components, well separated but in close proximity to each other, and finally by the front of the "developer." None of the "developer" appears in the component solutions which precede it.

The application of these principles of adsorption analysis to ion-exchange chromatography has been discussed by Samuelson (1953).

2. Partition

Partition chromatography was introduced by Martin and Synge in 1941 (*cf.*, Block and Bolling, 1951, p. 405 *et seq.*) as a means of separating acetylamino acids. In place of the normal solid adsorbent in column chromatography, an inert powder (precipitated silica) was used as a mechanical support for a stationary liquid phase (water). The column was run with a second liquid phase (such as chloroform-butanol) immiscible with the first. In this system the physical property which made separations possible was partition between the two liquid phases, rather than adsorption on the solid phase. In fact, free amino acids could not be well separated in this manner because of adsorption on the silica gel. For a particular solvent mixture, each acetamino acid moved down the column at a definite rate and could be collected as it emerged. The method was later developed by Gordon (1943) into a standard procedure for the determination of phenylalanine, leucine plus isoleucine, valine, alanine, proline, tyrosine and methionine in hydrolyzates from 25 mg. of protein.

Amino acids, without prior acetylation, were also separable by partition chromatography when raw potato starch was used as mechanical support for the water phase and 1-butanol was the immiscible solvent (Synge, 1944). A quantitative analytical method based on these observations was worked out by Stein (1949).

GENERAL METHODS

The principles of exchange, true adsorption and partition become interrelated in the practice of ion-exchange chromatography. In combination they are responsible for the high resolving power of the method. Even complex mixtures of closely related compounds such as are in protein hydrolyzates can thus be resolved completely. Different procedures have been elaborated for isolation and for quantitative analysis.

1. Isolation

Displacement Analysis: Partridge, in a series of papers, studied displacement chromatography on ion-exchange resins as a means of separating amino acids. This work is summarized in the systematic method of Partridge (1952a; 1952b) (*cf.*, Davies, 1950; Hagdahl, 1954ab; Kunin, 1951). Three resins were used: the cation exchangers, sulfonated cross-linked polystyrene and Zeo-Karb 215, and the strongly basic anion exchanger, Dowex 2. In a preliminary step the amino acid hydrochlorides from a yeast protein hydrolyzate were treated with active charcoal to remove tyrosine and phenylalanine which, if allowed to remain, behaved irregularly later in the procedure. The aromatic amino acids were recovered by elution with aqueous phenol-acetic acid and separated by fractional crystallization. The charcoal-treated filtrate was then fractionated as follows:

Primary Separation: First Stage. The mixture was adsorbed on a column of sulfonated polystyrene resin and displaced with dilute NaOH. Fractions of the eluate were collected by an automatic device. The early fractions contained the acidic and neutral amino acids and the later ones, the basic amino acids. Lysine and arginine were well resolved and required no further purification. The crude histidine fraction was adsorbed on Dowex 2 and displaced with dilute HCl. Some additional lysine was displaced first and later, pure fractions of histidine were obtained.

Primary Separation: Second Stage. The early fractions from the first stage were combined. The mixture was adsorbed on Zeo-Karb 215. The displacement developer was dilute NH_4OH. The effluent showed five fairly well defined bands of the following composition:

I. Pure aspartic acid.
II. Glutamic acid, serine, threonine.
III. Glycine, alanine.
IV. Valine, proline.
V. Leucine, isoleucine, methionine.

Band I required no further purification but there was minor overlapping in some of the other bands. Each of the mixed bands was passed through a column of Dowex 2, and displaced with dilute HCl. This effected final purification of each amino acid except that the pairs, leucine-isoleucine and serine-threonine, were not resolved. However, serine-threonine could be separated by displacing the mixture from a column of sulfonated polystyrene resin, using NaOH dissolved in aqueous acetone as displacement developer.

Cystine was present only in traces in the yeast hydrolyzate. In another experiment with egg albumin it was found that most of the cystine occurred in Band V, while some was present in the basic amino acid fraction. Cystine was readily isolated from these by simple crystallization.

This system was developed as a preparative method for the isolation of amino acids from quantities of protein ranging from 10 to 250 grams. Displacement analysis has an advantage over elution analysis in its higher capacity for a resin column of given size. Elution analysis, however, because of its higher resolving power, is more suitable for quantitative analysis and for isolation work on a small scale.

Elution Analysis: Hirs (1952, 1954a) developed two procedures for the isolation of small amounts of amino acids by ion-exchange chromatography and elution analysis. The first method (1952) using volatile buffers as eluting agents was applied to an acid hydrolyzate of 2.5 g. of bovine serum albumin. For complete separation of amino acids four columns of resin were required: Dowex 50 (NH_4^+) for the basic amino acids, Amberlite IR-4B (CH_3COO^-) for the acidic amino acids, and two columns of Dowex 50 (NH_4^+) for the neutral amino acids. Elution was effected with ammonium formate or acetate buffers in the range, pH 3 to 7.

Each of the well-resolved amino acids was isolated by removal of the buffer by sublimation and subsequent crystallization. All the amino acids (except methionine) were obtained in analytically pure form in an average yield of 66%.

The second method (Hirs, 1954a) is somewhat simpler, involving fewer steps and the use of a volatile acid as the eluting agent. An acid hydrolyzate of 70 mg. of bovine serum albumin was used. The amino acids were first chromatographed on Dowex 1-X8 acetate (a strongly basic anion exchanger containing quaternary ammonium groups attached to a styrene-divinylbenzene copolymer; X8 means that the resin contains 8% divinylbenzene and is a measure of the amount of cross-linking). In the chromatogram dilute acetic acid eluted Fraction *A* (containing most of the amino acids) then tyrosine, glutamic acid and aspartic acid, each well separated. Fraction *A* was taken to dryness to remove the acetic acid. The residue was dissolved in *N* HCl and rechromatographed on Dowex 50-X4 (H^+). Elution was begun with *N* HCl at 25° C. Serine, threonine, and glycine were obtained in well resolved peaks. The temperature was raised to 50° C. and, at the same time, the composition of eluting agent was changed gradually from 1 *N* to 4 *N* HCl by a mixing device.* Pure amino acid peaks were collected in the following order: alanine (plus ammonia), valine, proline, methionine, isoleucine, leucine, cystine, lysine, histidine, arginine and phenylalanine. Each amino acid was isolated by crystallization. Recoveries were quantitative with the following exceptions. Only 85% of the glutamic acid and 70% of the aspartic acid were recovered from the Dowex 1 column. Methionine presented a special case in this work and was not recovered quantitatively.

2. Quantitative Analysis

Starch Column Chromatography (Stein, 1949). As mentioned previously, partition chromatography on potato starch columns has been used successfully in amino acid analysis by Stein (1949). For quantitative analysis of the separated amino acids in the effluent fractions, an accurate spectrophotometric ninhydrin method was employed (Moore, 1948). Three chromatograms on starch columns were required for complete separation and determination of all amino acids and ammonia in a protein hydrolyzate. The starch columns were prepared with moist potato starch suspended in 1-butanol and then equilibrated with the solvent to be used in the run.

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* This consists of a 250-ml. chamber (a modified, ground joint wash bottle), equipped with magnetic stirrer, and filled with 1 *N* acid; 4 *N* acid is then fed in gradually. Details are given by Moore (1954a).

For the first chromatogram an aliquot of the amino acid hydrochlorides equivalent to about 2.5 mg. of protein, was added to the column, and the column was developed with *n*-butyl alcohol:*n*-propyl alcohol: 0.1 *N* HCl = 1:2:1 v/v. Fractions of the effluent were collected mechanically. The amino acids emerged in the following order: leucine plus isoleucine, phenylalanine, valine plus tyrosine plus methionine, proline, alanine plus glutamic acid, threonine, and aspartic acid. Only the proline, threonine and aspartic acid peaks were sharply resolved and suitable for quantitative analysis. The solvent was then changed to *n*-propyl alcohol:0.5 *N* HCl = 2:1 v/v which separated the remaining amino acids into pure peaks: serine, glycine, ammonia, arginine, lysine, histidine and cystine. The ten pure fractions from this chromatogram were analyzed.

A second chromatogram with another aliquot of the hydrolyzate developed with *n*-butanol:benzyl alcohol:water = 100:100:28.8 v/v as solvent yielded well resolved peaks of phenylalanine, leucine, isoleucine, methionine, tyrosine and valine. These were the first amino acid peaks to emerge and were suitable for analysis.

The final chromatogram was run with a third aliquot and *tert*-butyl alcohol:*sec*-butyl alcohol:0.1 *N* HCl = 2:1:1 v/v as solvent. A fore fraction containing the leucines, phenylalanine, valine, methionine and tyrosine was eluted prior to the emergence of proline. Pure fractions of glutamic acid and alanine were then collected and analyzed.

Each chromatogram was run with about 2.5 mg. of protein. β-Lactoglobulin and bovine serum albumin were analyzed by this method and excellent total nitrogen and weight recoveries of each protein in terms of known amino acids and ammonia were reported. The accuracy of the analytical method may best be judged by recoveries of 100 ± 3% for the individual components of synthetic mixtures of amino acids. Although the method has been in general use since its publication, the more convenient ion-exchange procedure of Moore (1951) superseded it. The resin columns employed in the latter method have higher resolving power than starch columns, and are not adversely affected by the presence of inorganic salts. The newer method is also faster.

Ion Exchange Chromatography (Moore, 1951)

(a) *Principle.* A mixture of amino acids (3 to 6 mg.) is added to a 0.9 × 100 cm. column of Dowex 50-X8 (Na^+).[*] The amino acids are eluted by a series of buffers of progressively increasing pH, from 3.4 to 11, at different temperatures. The effluent is collected by means of an automatic fraction cutter in 1-ml. portions in optically calibrated tubes. The amino acids emerge in a definite sequence, well separated from each other. Ninhydrin reagent is added to each tube for spectrophotometric determination of amino acid concentration. Summation of the quantities of amino acid found in the appropriate group of tubes gives the yield of each amino acid. Quantitative recoveries (100 ± 3% on the average) are obtained for all but the basic amino acids. These and ammonia are determined quantitatively with equal accuracy in a separate experiment on a 0.9 x 15 cm. column of the resin with buffers ranging from pH 5 to 6.8 at 25° C.

(b) *Equipment and Reagents.* (1) *Fraction Collector:* An automatic machine designed to collect successive fractions of effluent of known volume from the chromatograph column is required. A number of such machines are available commercially.[†] One, accommodating 200 tubes, and based on the original design of Stein (1948) is manufactured by the Technicon Company, Chauncey, New York. Also available from this source are convenient 50-tube aluminum racks for handling the tubes used both in the collector and spectrophotometer.

(2) *Spectrophotometer:* An instrument such as the Coleman junior or the Beckman Model B spectrophotometer, wherein direct readings of optical density in 18 x 150 mm. test tubes may be made, is needed.

(3) *Calibration of Test Tubes:* A matched set of at least 400 test tubes (18 x 150 mm., without lips) should be calibrated. Prepare a solution of methyl red in 0.03 *N* HCl of such strength as to give a reading at 525 mμ of 0.6 to 0.7 on the optical density scale against a water blank.[**] Add 5 to 10 ml. of the solution to each tube, and select only those tubes which read within 0.005 unit of the same optical density value. Mark permanently the side of the tube facing the

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[*] Mention of products or of apparatus in this chapter does not imply endorsement or recommendation of the Department of Agriculture over similar products or apparatus not mentioned.

[†] Literature references concerning the design of various types of fraction collectors may be found in an article by Schroeder (1954).

[**] Instead of the methyl red solution, the more stable solution of 0.12 *M* $CoCl_2 \cdot H_2O$ in 0.25 *N* HCl at a wave length of 515 mμ is preferable for these calibrations (Gordon, unpublished).

light source and align the tube in the same way each time it is read. Number the selected tubes with a glass marking tool.

Aluminum caps are convenient covers for the tubes during the course of analysis.

(4) *Boiling Water Bath:* This bath, for ninhydrin color development, should be large enough to hold a rack of 50 tubes immersed in vigorously boiling water to a depth of about 5 cm.

(5) *Constant Temperature, Circulating Water Bath:* This is required to maintain the temperature of the 100 cm. column at 37.5, 50 and 75° ± 0.5° C.

(6) *Pipetting Machine:* Although such a device is not essential, it saves much time in dispensing reagents. Suitable instruments are sold by the Baltimore Biological Laboratories, Inc., Baltimore, Md., and by the National Instrument Co., Baltimore, Md.

(7) *Buffers:* These may be prepared in quantity, adjusted if necessary with a little *N* HCl or *N* NaOH, and stored in the cold with thymol.

(a) Citrate buffer, 0.2 *M*, for ninhydrin solution. Dissolve 21.008 g. of citric acid ($C_6H_8O_7 \cdot H_2O$) in 200 ml. of *N* NaOH and dilute to 500 ml. with H_2O. The pH of the buffer when diluted with an equal volume of H_2O should be 5.0 ± 0.1.

(b) Buffer, pH 3.42 ± 0.01. To 500 ml. of the above citrate buffer (0.2 *M*) add 110 ml. of 1.0 *N* HCl and 390 ml. of H_2O. Prepare this buffer in 10 1. quantities. Before use, add 0.5 ml. of thiodiglycol and 1 ml. of BRIJ 35 solution per 100 ml. of buffer.[*]

(c) Buffer, pH 4.25 ± 0.05. To 500 ml. of citrate buffer (0.2 *M*) add 50 ml. of N HCl and 450 ml. of H_2O. Before use, add 0.5 ml. of thiodiglycol, 1 ml. of benzyl alcohol,[†] and 1 ml. of BRIJ solution per 100 ml. of buffer.

(d) Buffer, pH 5.0 ± 0.1. To 500 ml. of citrate buffer (0.2 *M*) add 500 ml. of H_2O. Before use, add 0.1 g. of disodium versenate,[**] 1.5 ml.

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[*] Thiodiglycol is added as an antioxidant to prevent losses of methionine. It may be obtained by the redistillation under reduced pressure of Kromfax solvent (Carbide and Carbon Chemicals Corp.) or of technical 2.2'– thiodiethanol (Eastman). BRIJ 35 (Atlas Powder Co., Wilmington, Del.) is a neutral, stable detergent, which permits faster flow during elution. Dissolve 50 g. of BRIJ 35 in 100 ml. of H_2O for the stock solution.

[†] Benzyl alcohol improves the resolution of tyrosine and phenylalanine.

[**] Analytical grade disodium versenate (Versenes Inc., Framingham, Mass.) containing negligible amounts of ninhydrin-positive impurities should be used. If versenate is not included in the buffers, low recoveries may be obtained after continued use of the 15 cm. columns.

of benzyl alcohol, and 1 ml. of BRIJ solution per 100 ml. of buffer.

(e) Buffer, pH 6.8 ± 0.03. To 500 ml. of 0.1 *M* Na_2HPO_4 add 450 ml. of 0.1 *M* $NaH_2PO_4 \cdot H_2O$. Before use, add versenate, benzyl alcohol and BRIJ as for preceding buffer.

(f) Buffer, pH 6.5 ± 0.05. Dissolve 42 g. of citric acid ($C_6H_8O_7 \cdot H_2O$) in 580 ml. of 1.0 *N* NaOH and dilute to 1 liter with H_2O. Before use add versenate, benzyl alcohol and BRIJ as for preceding buffer.

(8) *Ninhydrin:* The product sold by Dougherty Chemicals, Richmond Hill, New York, is sufficiently pure without recrystallization.

(9) *Methyl Cellosolve:* The reagent should be clear when mixed with an equal volume of water, and peroxide-free (test with 10% aqueous KI). Hamilton (1954) suggests that storage in the presence of zinc-copper couples will keep the reagent peroxide-free.

(10) *Ninhydrin Solution:* Dissolve 0.80 g. of reagent $SnCl_2 \cdot H_2O$ in 500 ml. of citrate buffer (0.2 *M*). Dissolve 20 g. of ninhydrin in 500 ml. of methyl cellosolve. Mix, and preserve the mixture under nitrogen. For fewer analyses, and especially if a pipetting machine is not available, it is also satisfactory to prepare only the minimum volume of ninhydrin solution required for daily use and to use a burette to measure out the solution for color development; in this case storage under nitrogen is not necessary.

(11) *Diluent Solution:* 1-propanol:water = 1:1 $^v/v$.

(12) *Resin:* Dowex 50-X8, minus-400 mesh grade, may be obtained from Technical Service and Development, The Dow Chemical Company, Midland, Michigan. One pound of the resin is sufficient for the preparation of several 100 cm. columns.

(13) *Chromatograph Columns:* Zechmeister-Cholnoky type, with ground joints and coarse sintered glass plates; glass tubing 0.9 cm. inner diameter, 1.5 to 2 mm. wall thickness; length of tubes: for 100 cm. resin column, 115 cm., and for 15 cm. resin column, 25 cm.

The long tubes are jacketed to permit temperature control by circulation of water from the constant temperature bath.* The short tubes are operated at room temperature.

(c) *Preparation of Resin Columns*

Wash one pound of Dowex 50-X8 (H^+) with 4 to 8 liters of 4 *N* HCl on a Buchner funnel with gentle suction. The final washes should be almost colorless. Wash twice with distilled water and

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* A convenient rubber centrifugal pump is manufactured by Schaar and Co., Chicago, Ill.

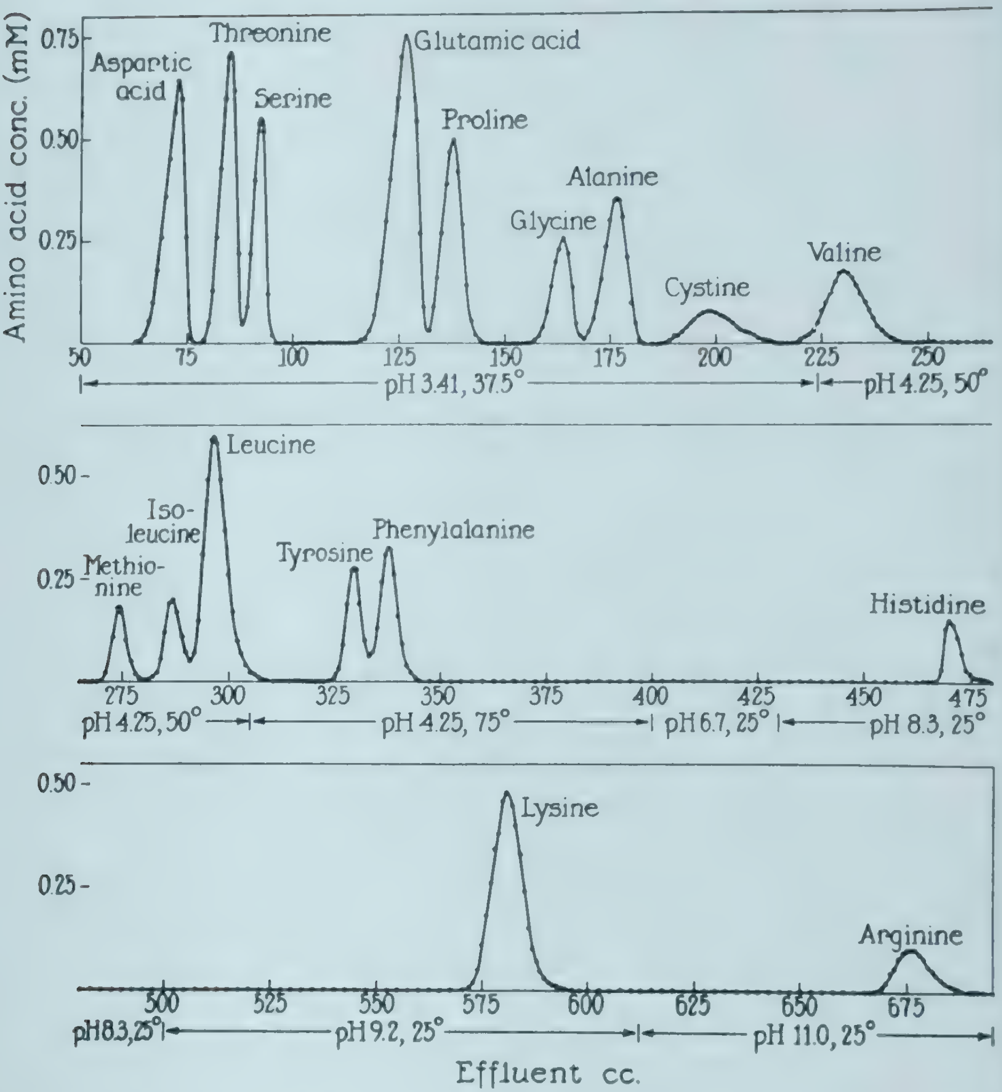

FIGURE 38. Separation of amino acids from a synthetic mixture simulating the composition of a protein hydrolyzate. The column of Dowex-50, 0.9 x 100 cm., was operated in the sodium form, with buffers of the pH and temperature indicated as eluants. A sample of about 6 mg. of amino acids was chromatographed. The position of an amino acid peak is reproducible, on the average, to better than 5%. (From Moore, 1951.)

then with 2 *N* NaOH until the filtrate is alkaline. Suspend the resin (Na^+) in about 3 times its volume of *N* NaOH and heat on a steam bath for about 3 hours with occasional shaking. Allow to settle for about ½ to 1 hour, decant the supernatant liquid, and add fresh, hot *N* NaOH. Repeat hot alkali treatment 5 times in all. The final washes are almost clear. Filter the resin, wash free of alkali and screen the wet resin through a 200 mesh sieve (74 μ openings) with the aid of a strong jet of water. Discard the large particles. Filter off the "through-200 mesh" resin and store as the moist sodium salt. The currently produced resin (1955) will give at least 50% yields of "through-200 mesh" resin. Moore (1954a) states that "through-200 mesh" resin gives elution curves superior to that shown in Figure 38 which was obtained with "through-120 mesh" resin. With the finer resin the leucine-isoleucine and tyrosine-phenylalanine separations are complete, and the cystine peak is sharper.

To prepare a column for use, wash some of the resin on a filter with a small amount of the pH 3.4 buffer (without detergent). Suspend the washed resin in about twice its volume of buffer. Allow to settle and remove bubbles by gentle stirring. Pour the slurry into the chromatograph tube in 5 portions of about equal volume (50 ml.), using a funnel with a bent, constricted tip to direct the flow of slurry against the side of the tube. Allow the first portion of resin to settle under air pressure of 10 to 15 cm. of mercury until there is no further drop in the height of the surface of the resin. Maintain the pressure until the liquid surface falls to within about 10 cm. of the resin surface. Then add a second portion of the slurry as before. Continue the additions of resin until a column of packed resin, 100 cm. in height, is obtained. If the top surface of the resin is irregular, stir it with a little supernatant buffer and allow it to settle again.

Prepare the 15 cm. column similarly in one or two sections.

Attach a separatory funnel (about 300 ml. capacity) as a reservoir for appropriate buffer to the column with a length of plastic tubing so that the funnel may function as a leveling bulb. Run about 100 ml. of buffer through the column. It is then ready for use.

(d) *Procedure for Photometric Ninhydrin Method* (Moore, 1948, 1951)

To 1 ml. of amino acid solution at about pH 5.0, add 2 ml. of ninhydrin solution. Cap the tube and mix by shaking. Heat in *vigorously boiling* water bath for 20 minutes. Allow to cool for a few minutes and add 5 ml. of diluent solution. Shake well for 1 minute. Read in the spectrophotometer beginning at about 15 minutes after removal from the bath, using 440 mμ for proline and hydroxyproline and 570 mμ for all other amino acids. Read reagent blanks against the diluent. Select average reagent blank, set instrument to zero on this blank, and read unknown. The reagent blank reading should be about 0.15 to 0.20 on the optical density scale. Analyses should be run in groups of not more than 50 tubes to permit readings to be completed within 1 hour after the heating period; during this time readings remain essentially constant.

When optical density values at 8.0 ml. total volume are too high for accurate reading, further additions of one, two or three 5 ml. portions of diluent solution are made, the average reagent blank is diluted likewise, and readings are taken at 13, 18 or 23 ml. If readings at 23 ml. are still too high, aliquots of unknown and blank are pipetted into other calibrated tubes and further dilution is made.

Leucine Calibration Curve: On a molar basis the amino acids do not give the same color yield in the ninhydrin reaction. However, only one calibration curve is required because accurate color yields relative to leucine have been established. Relative to the leucine color value at 570 mμ taken as 100, the color yields per mole for other amino acids are: Hydroxyproline 3; aspartic acid 93; threonine 92; serine 97; glutamic acid 105; proline 5; glycine 101; alanine 99; cystine 108; valine 100; methionine 100; isoleucine 100; tyrosine 91; phenylalanine 91; tryptophan *ca.* 72; histidine 104; lysine 112; ammonia *ca.* 98; and arginine 100. Spectrophotometer readings taken at 440 mμ for proline (and for hydroxyproline, if present) are first converted to "micromoles leucine" using the leucine calibration curve at 570 mμ employed for the other amino acids; these values are then multiplied by 3.7 to give micromoles of proline and by 7.2 to give micromoles of hydroxyproline.

Prepare a calibration curve, plotting micromoles of leucine against optical density, with a sample of leucine of established purity. Use aqueous solutions of the amino acid at several different levels of concentration, within the range 0.05-0.20 micromoles of leucine per 1 ml. aliquot. This is a convenient range for ninhydrin color development by the above procedure in a total volume of 8 ml. Once the standard curve has been confirmed by replicate experiments, a standard table relating optical density to micromoles of leucine may be prepared and from these figures further tables may be calculated at total volumes of 13, 18 and 23 ml. for higher concentrations of amino acid.

(e) *Operation of 100 Cm. Columns*

With the prepared column in place above the fraction collector,* begin circulating water at 37.5° C. through the jacket about an hour before the start of a run. Heat to boiling about 300 ml. of buffer of pH 3.42 to free it of dissolved air, pour the warm buffer into the separatory funnel, and cover the solution with a thin layer of mineral oil. Add the sample of amino acid solution to the column with a 1 or 2 ml. pipette with bent tip so as not to disturb the surface of the resin. When the sample is drained into the resin, wash down in the same way with three successive 0.3 ml. portions of buffer, and finally layer about 2 ml. of the buffer above the surface of the resin. Do not allow the surface of the resin to dry out during these operations. The pH of the amino acid solution should be more acid than that of the buffered column, about 2.5 to 3.0 being optimal. Usually, a charge of about 5 mg. of total amino acid mixture is introduced for a chromatogram but several times this quantity is permissible and is recommended for more accurate determination of amino acids present in small concentration.

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* If the collector is of the Technicon type, with the tubes housed in an enclosed chamber, it is well to circulate a slow stream of ammonia-free air through the chamber; shallow dishes containing citric acid solution may also be used on top of the rack to minimize absorption of ammonia by the acidic eluent.

Connect the buffer reservoir and adjust the flow rate of solvent through the column to about 4 ml. per hour by adjusting the height of the separatory funnel or by using slight air pressure (*cf.*, Mueller, 1949). Collect the effluent in 1 ml. fractions.

Figure 38 shows that both buffer and temperature are changed at the beginning of the valine peak (about 225 ml.). Before this point is reached, it will have been possible to develop and analyze the ninhydrin color of tubes 100-150. Note the position of the yellowish-red proline peak. For example, tube 135 may have contained the maximal amount of proline. Multiply 135 by the factor 1.63 to give 220 ml. Use this figure as a better estimate of the start of the valine peak. At this point, change the setting of the temperature regulator in the water bath to 50° C. Replace the buffer pH 3.42 in the reservoir with boiled buffer of pH 4.25. Allow the surface of the 3.42 buffer in the column to fall to about 1 mm. above the resin surface, then with bent pipette add about 2 ml. of 4.25 buffer, and connect the reservoir of fresh buffer. As the temperature of the column rises, maintain the desired flow rate by lowering the pressure. Change the temperature to 75° C. when another 75 ml. have been collected (at about 295 ml.), and again adjust the flow rate. Usually, the chromatogram is stopped at about 385 ml. because the basic amino acids cannot be recovered quantitatively. Allow the column to cool to room temperature before closing the stop-cock of the separatory funnel.

If it is desired to complete the chromatogram as shown in Figure 38, consult the original paper of Moore (1951) for preparation of buffers and procedure. Two-dimensional paper chromatograms should be carried out to establish the presence of all the components of an unknown mixture. Because of the limited resolving power of the 15 cm. column, it cannot be relied upon to differentiate ornithine from lysine, hydroxylysine from histidine, or glucosamine from tryptophan.

At the end of the chromatogram, clean the column by running through about 100 ml. of 0.2 *N* NaOH containing BRIJ. Then run through 100 ml. of pH 3.42 buffer and the column is ready for use.

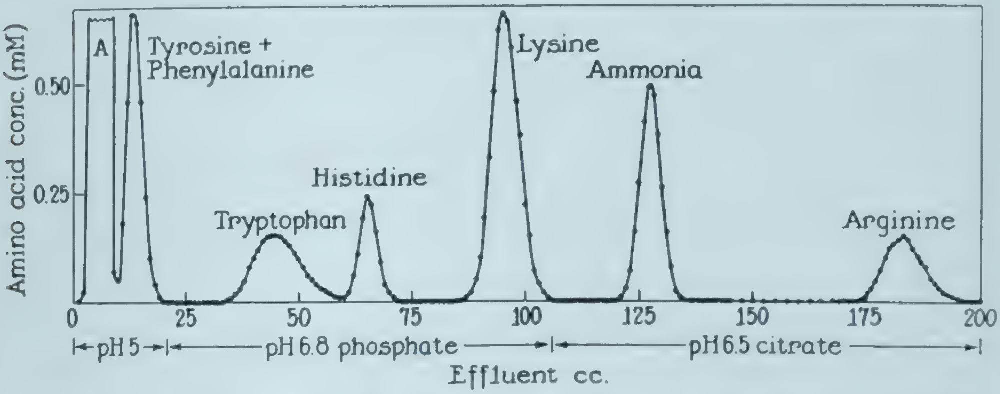

FIGURE 39. Separation of the basic amino acids on a column of Dowex-50, 0.9 x 15 cm. The column was operated in the sodium form at 25° C. with the buffers indicated. A sample of about 6 mg. of amino acids as in Figure 38 was employed. The large peak, A, comprises all the amino acids emerging before tyrosine in Figure 38. (From Moore, 1951.)

If the surface of the column becomes clogged at any time, remove the top cm. of resin and replace it with fresh resin. Never allow the resin to dry out.

(f) *Operation of 15 Cm. Columns*

Run the chromatogram at room temperature and as shown in Figure 39. Heating of the buffers is not required. Before use, run about 25 ml. of pH 5 buffer through the column. Add the load of amino acids in 1.0 or 2.0 ml. of solution of about pH 4. For the desired flow rate it may be necessary to lower the buffer reservoir and to operate it at negative pressure. Make the changes of buffer as indicated in Figure 39. If histidine is not well separated from lysine, adjust the pH of the 6.8 phosphate buffer to 6.85 or 6.90. Clean the column before reuse with about 25 ml. of 0.2 *N* NaOH plus BRIJ.

(g) *Analysis of Collected Fractions*

Develop the ninhydrin color in 50 tubes at a time. First adjust the pH of each fraction to about pH 5 with one or two drops of HCl or NaOH as required. The following figures give the approximate amounts required to adjust 1 ml. of buffer to pH 5: buffer 3.42, 0.1 ml. of *N* NaOH; buffer 4.25, 0.05 ml. of *N* NaOH; buffer 5.0, none; buffer 6.8, 0.05 ml. of 0.5 *N* HCl; buffer 6.5, 0.05 ml. of 2 *N* HCl. Determine the proper amount and strength of acid or alkali to be added by titrating aliquots of the different buffers before running the chromatogram. By calibrating the size of drops from a slowly dripping burette and by adjusting the strength of acid or alkali, establish the required number of drops for 1 ml. of each buffer.*

The hold-up volume of the 0.9 x 100 cm. columns is about 22 ml., but the more acidic buffers have a significant retardation volume. Thus, if the change of buffer to 4.25 is made at tube number 220, buffer 4.25 will not emerge from the column until tube number 255 (35 ml. later). Therefore, for the pH adjustment, change from 2 drops of alkali to 1 drop at tube 255. For the 15 cm. columns, make the changes in adjustment of pH, 4 ml. after the change of buffer.

Add ninhydrin and develop color as described. In making the optical density readings it is important to choose the proper blank against which amino acid concentration is read. In the aspartic acid-proline region (Figure 38) choose a representative blank from the valleys in the curve. For the glycine and alanine peaks, select a blank after the proline peak. The curve may or may not return to the base line both before and after the cystine peak. Choose the blank for cystine and valine after the valine peak. The blank reading usually changes somewhat with the emergence of the pH 4.25 buffer. Select the blank for methionine, isoleucine and leucine after the leucine peak.

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* The development of a modified ninhydrin reagent with greatly increased buffering capacity makes possible analysis of the effluent fractions without adjustment of pH. If the modified reagent is used, the detailed procedure in the original paper (Moore, 1954b) should be followed. It will be noted that there are important points of difference in the procedure with the newer reagent: use of 1 ml. of reagent, 15 minute heating period, ethanol-water as diluent, shaking required to remove reddish hydrindantin color, new color factors for amino acids relative to leucine, etc. The modified reagent is prepared as follows:

4 N Sodium Acetate Buffer (pH 5.51 ± 0.03). Dissolve 2720 gm. of Na O Ac • 3 H_2O in 2 liters of H_2O on a steam bath. Cool to room temperature, add 500 ml. of glacial acetic acid and then H_2O to a volume of 5 liters. Final adjustment of pH, if necessary, is made with solid NaOH or glacial acetic acid. Store in the cold; preservative is not needed.

Reagent Solution. Dissolve 20 gm. of ninhydrin and 3 gm. of hydrindantin (Dougherty Chemicals) in 750 ml. of methyl cellosolve with gentle stirring. Add 250 ml. of pH 5.5 buffer. Transfer the resulting reddish solution immediately to a dark glass reservoir bottle and store under nitrogen. Prepare not more than a week's supply of reagent at one time.

Only the most alkaline buffers (pH 8.3, 9.2 and 11.0) used for the elution of basic amino acids in the 100 cm. column procedure (Figure 38) would still require adjustment of pH prior to color development with this reagent.

In the 15 cm. column runs (Figure 39) the blank reading increases about 0.15 optical density unit when the pH 6.5 buffer emerges. Choose a blank for the ammonia peak just after the peak rather than before it. The blank usually falls again to its normal value before arginine emerges.

(h) *Calculation of Results*

Since the leucine calibration curve is plotted for volumes of 8.0 ml., volume corrections may be required in the calculation of results. Although the fraction collector is set to deliver samples of 1.0 ± 0.1 ml., it is advisable to spot check its performance by weighing every tenth tube in a run before and after a fraction has been collected. An approximate volume correction can then be made for any deviation from 1.0 ml. In addition, a correction may be made for the volume of acid or alkali used to adjust the solutions to pH 5. Because optical density readings are made at a minimal volume of 8.0 ml., these volume corrections are important only when maximum accuracy is sought.

Corrected optical densities are converted to "micromoles leucine" and the values for each peak are integrated or the sum of all the readings is used. Use of the appropriate color yield then converts these figures into micromoles of amino acid.

(i) *Preparation of Samples for Chromatograms*

Alanine-Cystine-Valine Mixture: The order and position of amino acid peaks emerging from the column, while quite reproducible under the conditions specified, are rather sensitive to changes of pH and ionic strength of the buffers. Of particular importance in this connection is the pH of the 3.42 ± 0.01 buffer. The position of the cystine peak varies markedly with small changes in pH of this buffer. At pH 3.47 cystine and alanine emerge almost together and at pH 3.37 cystine begins to overlap valine. It is necessary, therefore, to check the performance of the 3.42 buffer before use in analysis.

Prepare a solution in dilute HCl of 4 micromoles of alanine, 6 micromoles of valine, and 2 micromoles of cystine per ml. Mix 1 ml. of the solution with 1 ml. of pH 3.42 buffer and add 1 ml. of the mixture to the 100 cm. column. Run the chromatogram at 37.5° C. with the 3.42 buffer until valine is eluted (about 250 ml.). If the results show the peaks to be separated poorly, adjust the pH so that the cystine peak will be better positioned midway between the alanine and valine peaks, and repeat the experiment.

Amino Acid Mixture: It is important to run preliminary chromatograms with known mixtures of pure amino acids in order to establish a routine procedure whereby the reproducibility and accuracy inherent in the method can be realized. Unknown mixtures should then be analyzed in precisely the same way.

Prepare accurately a known mixture of amino acids and NH_4Cl simulating the composition of a protein hydrolyzate. Tryptophan may be omitted because the present method was developed for acid hydrolyzates. Cysteine may also be omitted because, if present, it is gradually oxidized and does not give a peak. The amounts of the 17 amino acids and NH_4Cl to be weighed out are determined by the following considerations. A total of about 1 g. of amino acids are dissolved in 1.5 ml. of 6 *N* HCl and made to 10 ml. with water. This solution may be stored in the cold. An aliquot of the solution is diluted 1 to 10 with pH 3.42 buffer for the 100 cm. column, or with pH 5.0 buffer for the 15 cm. column. A 0.5 ml. aliquot of the resulting solution will then contain about 5 mg. of total amino acids, a quantity suitable for analysis. The 5 mg. of total amino acids should include 0.1 to 0.5 mg. of each amino acid.

Recoveries of 100 ± 3% should be obtained for the majority of amino acids and ammonia. The recovery of glutamic acid is consistently about 97%, presumably because of slight conversion to pyrollidone carboxylic acid by the acidic solvents; a correction factor is used to compensate for this loss. Occasionally, slightly low recoveries (96%) of cystine and methionine are encountered.

(j) *Protein Hydrolyzates* (*cf.*, Chapter II)

With the development of methods for the precise analysis of mixtures of amino acids, increasing attention has been devoted to the conditions employed for the hydrolysis of purified proteins. It has been recognized that ordinary acid hydrolysis (6 *N* HCl, 16 to 24 hours, 100-120° C.) does not always liberate completely certain amino acids, such as valine and isoleucine, from resistant peptide linkages. It is also true that these conditions usually bring about complete destruction of tryptophan and variable decomposition of serine, threonine, cystine and, sometimes, other amino acids. For the determination of tryptophan, of course, some independent method of analysis, may be employed (*cf.*, Block and Bolling, 1951). In the case of the other amino acids, current practice is to use at least two different periods of hydrolysis, for instance, 20 and 70 hours. Comparison of the analytical results provides information about both liberation and destruction of amino acids and permits closer estimates of the true amino acid composition of the protein. (Harfenist, 1953; Smith, E. L., 1954a, 1954b; Hirs, 1954b; Gordon, 1955.)

Hydrolyzates for analysis by the method under discussion should be prepared by heating with HCl, the excess acid should be removed by evaporation *in vacuo*, and the amino acid hydrochlorides should be mixed with pH 3.42 buffer before addition to the 100 cm. column or with pH 5.0 buffer for runs in the 15 cm. column. Usually, a 1 ml. aliquot of the hydrolyzate is mixed with 1 or 2 ml. of buffer, and a 1 or 2 ml. sample is added to the column. The following points may be considered before a more specific procedure for hydrolysis is adopted. It has been recommended that proteins be hydrolyzed in 6 *N* HCl redistilled in glass; that a large excess of acid (100 to 200 times the quantity of protein) be used to reduce humin formation; and that an even larger excess of acid be used in the presence of carbohydrate (Jacobsen, 1949; Stein, 1948; Schram, 1953; Dustin, 1953a,b). Hydrolysis is often carried out in evacuated, sealed tubes with adequate control of the temperature (Harfenist, 1953; Smith, E. L., 1954a; Hirs, 1954b), although in the case of crude proteins, hydrolysis under reflux is preferable (*cf.*, Chapter II). Results may be based on dry weight of protein hydrolyzed or on determinations of nitrogen in the hydrolyzate.

APPLICATIONS OF THE METHOD

1. Difficulties

The ion-exchange method (Moore, 1951) has been applied without major modification to the analysis of different proteins in many laboratories. The chief difficulty encountered in its use, insufficient resolving power in the cystine, glutamic acid-proline, and phenylalanine-tyrosine regions of the curve, may probably be attributed to the lack of uniformity of commercial samples of the resin. The continued collaborative efforts of the authors of the method and the manufacturers of the resin to provide more rigid specifications for particle size and degree of cross-linking may well help to overcome this difficulty (*cf.*, Moore, 1954a, footnote 1).

Even though some samples of resin used in the past years have not been fully satisfactory in resolving power, they have always yielded the same sequence of amino acids when eluted in the prescribed manner, and it has been possible to resort to various expedients to obtain usable results. For example, in analyses of insulins, Harfenist (1953) found that it was necessary to apply the Chinard acid ninhydrin method (1952) in the proline region for more accurate determination of this amino acid; that cystine could not be determined chromatographically; and that independent estimations of tyrosine were required to obtain values for phenylalanine from the combined tyrosine-phenylalanine peak. In analyses of prothrombin Laki (1954) used independent methods for tyrosine and phenylalanine, and determined cystine-cysteine chromatographically as cysteic acid after prior oxidation with performic acid. Similar supplementary procedures for the aromatic amino acids and cystine were required in analyses of muscle proteins (Kominz, 1954). Dual readings at 570 and 440 mμ were made for overlapping peaks of glutamic acid and proline in analyses of myoglobin and hemoglobin by Rossi-Fanelli (1955).

Poor resolution of the pairs glutamic acid-proline and tyrosine-phenylalanine occurs only occasionally and can be dealt with when it does. Spreading of the cystine peak is more serious. Not only is poor resolution of cystine sometimes encountered with synthetic mixtures of amino acids, but additional spreading of the peak occurs in protein hydrolyzates because of racemization (Hirs, 1954a, 1954b). Complicating the situation still further is the variable destruction of cystine during hydrolysis, especially in the presence of carbohydrate (*cf.*, Chapter II). For these reasons, determination of cystine by some other method is usually necessary. Often, performic acid* oxidation of the protein, prior to acid hydrolysis, is carried out with a twofold purpose: to permit estimation of cystine in the form of cysteic acid, which emerges unretarded at the hold-up volume (about 22 ml.) of the 100 cm. column (Laki, 1954; Smith, E. L., 1954a); and to permit more accurate definition of the alanine and valine peaks unobscured by cystine (Gordon, 1955). Conditions for the conversion of cystine to cysteic acid, and chromatographic procedures for the more certain identification and estimation of cysteic acid have been reported (Schram, 1954; Partridge, 1955).

The occasional low recoveries of methionine from known mixtures, presumably due to oxidation despite the routine use of thiodiglycol as an antioxidant in the eluting buffers, has been mentioned. Some oxidation of methionine may also occur during hydrolysis, especially in the presence of carbohydrate, with formation of methionine sulfoxide. When this happens, a sharp peak of the sulfoxide emerging just ahead of aspartic acid at pH 3.42 can be detected in chromatograms. The ninhydrin color of the sulfoxide is 102 relative to leucine (Schram, 1953). If the peak of sulfoxide is quantitatively significant, the methionine value can be corrected accordingly. This has been done in analyses of foods, such as cassava meal (Close, 1953a), barley, hay, and linseed presscake (Dustin, 1953b), cottonseed protein (Adriaens, 1954), human milk (Soupart, 1954), and also in analyses of virus protein (de Fremery, 1955).

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* Oxidation of cystine and cysteine to cysteic acid and methionine to methionine sulfone with peracetic acid in aqueous solution is preferable to performic acid (Block, unpublished).

Another difficulty, which has been troublesome at times, is variation in the density of ninhydrin color in the blank tubes. This is especially important when an amino acid is present in low concentration and it is essential for accurate work to choose a representative blank against which readings the peak are to be made. Variations in the blank can arise from absorption of ammonia from the air (Fraser, 1953) and from the presence of ammonia in reagents (Rossi-Fanelli, 1955). Variations in the blank may also be due to incomplete hydrolysis of a protein and the emergence of minor peaks of residual small peptides; longer periods of hydrolysis are required in this case (Smith, E. L., 1954a). Minor peaks in chromatograms of unknown mixtures are not necessarily artifacts and if it is desired to identify these or other peaks by paper chromatography the reader is referred to the papers by Stein (1953), E. L. Smith (1954a), and the book by Block, Durrum and Zweig (1955).

The ninhydrin color yields of amino acids relative to leucine have been checked by Moore (1951), E. L. Smith (1954a) and presumably by other users of the method. Occasionally, however, other values such as 90 for ammonia (Harfenist, 1953), 97 to 99 for glutamic acid and 97 to 98 for glycine (Simmonds, 1954), and 107 for lysine (Gordon, 1955) have been reported. The importance in this connection of running chromatograms of known mixtures, under the specific conditions employed by each investigator, is obvious.

Poor recovery (87%) of histidine from known mixtures was reported by Harfenist (1953). Reproducible determinations of arginine could not be obtained in analyses of virus protein (de Fremery, 1955). No explanations have been advanced for these observations.

In analyses of gelatin for ornithine by the 15 cm. column procedure Hamilton (1954b) found it necessary to change the pH, concentration, and sequence of some of the eluent buffers to give a more satisfactory resolution of histidine from hydroxylysine and, particularly, of ornithine from lysine. The same procedure was used for determination of hydroxylysine in collagen (Hamilton, 1955). The complete amino acid analysis of soluble proteins derived from elastin by Partridge (1955) included determination of hydroxyproline. This amino acid emerges well ahead of aspartic acid from the 100 cm. column at pH 3.04; because of the weak ninhydrin color yielded by hydroxyproline, color density was read against a separate calibration curve prepared with hydroxyproline itself. The use of the Troll (1953a) ninhydrin method for the estimation of hydroxyproline following chromatographic separation has also been proposed (Rogers, 1954; *cf.*, Chapter III).

2. Comments

The statements of Moore (1951), concerning the accuracy of the method when applied to known mixtures, have been substantiated

TABLE XI

Amino Acids Found in α-Lactalbumin Hydrolyzates*

Amino Acid	*Time of Hydrolysis*					
	20 hrs.		*70 hrs.*		*140 hrs.*	
	H-5†	*H-6*	*H-7*	*H-8*	*H-9*	*H-11*
Aspartic acid	—	18.45	18.89	18.80	18.45	
Threonine	5.43	5.39	5.54	5.63	5.51	
Serine	4.70	4.70	4.73	4.88	4.79	
Glutamic acid	12.61	12.73	13.00	13.01	12.91	
Proline	1.52	1.64	1.53	1.48	1.43	
Glycine	3.07	3.12	3.25	3.23	3.32	3.24
Alanine	2.01	2.13	2.13	2.10	2.34	2.15
Cystine	4.37	5.60	3.58	4.33	3.05	6.66
Valine	3.20	3.25	4.60	4.52	4.89	4.62
Methionine	0.91	0.92	0.90	0.95	0.95	
Isoleucine	5.41	5.53	6.78	6.53	7.06	6.82
Leucine	11.09	11.44	11.67	11.54	11.93	11.43
Tyrosine	5.20	5.30	5.02	4.83	4.47	
Phenylalanine	4.36	4.27	4.47	4.62	4.64	

* The amino acid values are given as g. of amino acid yielded by 100 g. of anhydrous, ash-free protein.

† H-5 through H-11 designate individual hydrolyzates; H-11 was prepared after oxidation of the protein with performic acid.

Other comments: The figures for valine and isoleucine show that at least 70 hrs. of hydrolysis are needed for maximal yields of these amino acids. The yield of tyrosine decreases with time of hydrolysis. The figures for cystine illustrate the difficulties encountered in determination of this amino acid; the value of 6.66%, estimated as cysteic acid but expressed as cystine, is in fair agreement with independent determinations (6.4 ± 0.1%). The stability of serine and threonine in these experiments is noteworthy (Gordon, 1955).

in other laboratories. The reproducibility of results, when the procedure is applied to protein hydrolyzates, may be illustrated by some analyses of α-lactalbumin shown in Table XI (Gordon, 1955) and by similar tables for carboxypeptidase and papain (Smith, E. L., 1954a, 1954b).

Other proteins, not mentioned before, and some polypeptides which have been analyzed by ion-exchange column chromatography are presented in Part II.

Piez (1955) using a 100 x 0.9 cm. column of Dowex 50 at 50° C. and 6 ml. per hr. with 0.37 *M* citrate buffer at pH 3.1 (0.25 *N* sodium citrate) continuously varied by the addition of 0.25 *N* NaOH to give a ratio of flow rates in and out of the reservoir of 1:5.7 for 170 ml. and then a ratio of 1:10, found evidence of separation of isotopic (C^{14}) amino acids from their nonisotopic analogues.

3. Analysis of Amino Acid Solutions Not Obtained From Protein Hydrolyzates

For particular application of their procedures to mixtures of amino acids and related compounds more complex than protein hydrolyzates, Moore (1954a, 1954b; *cf.*, Partridge, 1952a; Sober, 1954; Thompson, 1953) has reported modifications of both the chromatographic separation and of the photometric ninhydrin method. A longer column (150 cm.), a 4% cross-linked resin (Dowex 50-X4), and an eluant of continuously changing pH and ionic strength are used to obtain a system with higher resolving power. A single chromatogram resolves 50 components almost completely. Modifications of the ninhydrin reagent include a fivefold increase in buffer strength to obviate the need for pH adjustment of the eluant, and replacement of stannous chloride by direct addition of hydrindantin. With the new method, analyses have been made of the free amino acids of blood plasma (Stein, 1954), tissue extracts (Tallan, 1954), and also of protein hydrolyzates (*cf.*, Part II). The chromatographic separations with Dowex 50-X4 are said to be more difficult to standardize than those with Dowex 50-X8.

4. An Automatic Procedure

A simplified chromatographic separation of amino acids together with an automatic recording apparatus for measuring ninhydrin color density have been described in a recent abstract (Spackman, D. H., Stein, W. H., and Moore, S., Federation Proceedings, *15*:358 (1956)). Two columns packed with fine mesh sulfonated polystyrene resin are required for complete quantitative amino acid analysis of a protein hydrolysate but only a single temperature (50° C.) and three eluent buffers are used, and the analysis is complete in 20 hours. The influent buffer is pumped through the column at a constant rate (up to 30 ml. per hour) by a stainless steel pump. The effluent from the column emerges in 1 mm. capillary tubing where it is mixed with a stream of ninhydrin reagent delivered at half the rate of the effluent by a second pump. The mixture passes through a 50 ft. spiral of 1 mm. -bore Kel-F tubing immersed in a boiling water bath where the ninhydrin color is developed. The optical density of the resulting solution is measured continuously as it flows through a cylindrical glass cell by three photometer units. Two of these measure densities at 570 mμ and the other at 440 mμ. The effective diameter of the glass cell is reduced from 2.2 to 0.8 mm. as it passes through one of the units measuring at 570 mμ in order to extend the range of the instrument when the optical density exceeds 1.3 with the larger cell. The optical density measured by each photometer is recorded by a Leeds and Northrop three-point recorder. The accuracy is 100 ± 3%.

CHAPTER VII

The Requirements for Amino Acids

By **Herman J. Almquist, Ph.D.**

ESTIMATION OF AMINO ACID REQUIREMENTS

The large number of investigations in this field precludes an attempt to review all contributions in this monograph. Rather, an effort has been directed toward including recent papers giving newer information, refinements in technique and references to the literature, which will assist the student to follow along any path of particular interest.

Several methods have been used in the estimation of protein and amino acid requirements of animals. These may be described briefly as procedures based upon 1) amino acid composition of the dietary proteins, 2) growth of young animals, 3) efficiency of diet or of nitrogen utilization, 4) nitrogen balance, 5) protein repletion or restoration of tissues of protein-depleted adult animals, 6) analysis of the carcass for amino acids, and 7) determination of minimum intakes in proteins of apparently adequate diets.

1. Chemical (Amino Acid) Methods

"The construction of tissue proteins is an 'all or none' proposition, in the sense that the supply of amino acids for synthetic purposes is utilized only to the extent permitted by that amino acid available in the least amount with reference to the requirements of the protein pattern being followed. The amino acid supply does not appreciably modify the pattern of synthesis in any tissue, and incomplete or abnormal proteins are not formed to better utilize the amino acid supply presented to the tissues by the digestive tract" (Mitchell, 1954b; *cf.*, Milligan, 1951; Block, 1944a).

As a result of numerous investigations the amino acids may be classified into three groups (Block, 1944b) as given in Table XII. Arginine is classified as semi-indispensable because, while it can be synthesized to some extent by the growing rat, the rate of synthesis, from proline and glutamic acid and possibly other compounds, is not sufficient for the support of maximum growth. Tyrosine is formed from phenylalanine. However, since tyrosine is a usual constituent of tissue proteins, the dietary requirement for phenylalanine is reduced by the presence of tyrosine up to the amount required to cover the tyrosine needs of this organism. Cystine is formed from dietary methionine and serine; therefore, dietary cystine will spare, at least, an equivalent quantity of methionine (Grau, 1943; Block, 1949a).

On the basis of his own work and that of others Mitchell (1954a, 1954b) expressed the concept that the amino acid requirements of the growing animal are determined in the last analysis by the amino acid composition of the tissue proteins formed during growth, superimposed upon the amino acid requirements for replacement of the endogenous losses of nitrogenous material, i.e., for maintenance of the *status quo*.

TABLE XII

NUTRITIVE CLASSIFICATION OF THE AMINO ACIDS FOR THE GROWING RAT (Block, 1944b)

Essential (Indispensable)	*Semi-indispensable*	*Nonessential (Dispensable)*
Histidine	Arginine*	
Lysine		
Phenylalanine	Tyrosine	
Tryptophan		
Methionine (+ Serine or Glycine)	Cystine	
Threonine	Glycine*	Serine*
Leucine		Glutamic acid
Isoleucine		Aspartic acid
Valine		Alanine
		Proline
		Hydroxyproline

* Arginine and glycine (or serine) are essential amino acids for the chick.

The Qualitative Biological Evaluation of Proteins From Their Amino Acid Composition. Apparently the first serious attempt to evaluate the nutritive potency of proteins from their amino acid composition was carried out by Mitchell (1946) and Block (1946-47). The idea was to judge the nutritive adequacy of a protein by the severity of its limiting essential amino acid deficiency. The standard protein source was whole egg which had been shown to be nearly perfectly utilized in digestion and in metabolism for the growing rat, the mature rat, the dog, and for adult man. Furthermore, it had been shown that whole egg protein was not improved for the growing rat by supplementation with any essential amino acid except lysine, which induced a 3% increase in body weight in 28 days (*cf.* Mitchell, 1954b). The absence of any nutritionally unavailable essential amino acid in whole egg proteins is shown by the fact that substitution of whole egg for starch at low levels in a nitrogen-free diet fed to rats does not appreciably increase the output of urinary nitrogen (*cf.* Mitchell, 1954b).

On this basis Mitchell (1946) and Block (1946-47) compared the contents of the essential amino acids in single proteins and in the mixed proteins of foods with those of egg by computing the percentage deviation for each amino acid. The nutritive value of the protein was expressed as a "chemical score," equal to the greatest percentage deficit in an essential amino acid in the protein or protein mixture under scrutiny. The *chemical score was taken as 100 minus the greatest percentage deficit;* in other words, the smallest percentage of an essential amino acid based on its content in egg proteins.

The chemical score measures the value of proteins for growth only, since it assumes that the complete lack of an essential amino acid renders a protein completely unavailable for the synthesis of body proteins. For example, if lysine is the limiting amino acid of a food protein that contains only one-half of the quantity of lysine in egg proteins, then for purposes of growth only, the utilization of all other essential amino acids would be limited to 50% of the amount present in the standard. However, the exact extent of general limitation is not known; presumably it would be a function of the ratio of amino acid nitrogen used for growth to that used for maintenance which in turn would depend upon the level of protein in the diet.

Extensions of the chemical score method (Mitchell, 1946; Block, 1946-47) have been developed by Kühnau (1949), Oser (1951), Mitchell (1954a, 1954b) and others. These methods differ from the "chemical score" method in that the relative quantities of all the essential amino acids enter into the calculations. For the sake of brevity only the "modified essential amino acid index" of Mitchell (1954a, 1954b) will be given in detail. Table XIII gives the calculation of the modified essential amino acid index of lactalbumin using egg proteins as the standard. Note that arginine is not used in the calculation but that tyrosine and cystine are employed. Note also that in each case where the test protein contains more of the essential or semi-essential amino acid than the standard, the excess is neglected. Although there is a higher correlation of the essential amino acid indices and the biological values of proteins than between the chemical scores and the biological values, it is our opinion that in the case of proteins that are markedly deficient in only *one* essential amino acid, the *chemical score method* of evaluation is preferable. On the other hand, when one wishes to calculate the biological value of mixed diets which are but slightly deficient in a number of essential amino acids, the *modified essential amino acid index* is more accurate. The reader is urged to study the excellent papers by Mitchell (1954a, 1954b) for a further detailed discussion of the dependence of the biological value of food proteins upon their amino acid content.

TABLE XIII

CALCULATION OF THE BIOLOGICAL VALUE OF LACTALBUMIN BY THE MODIFIED ESSENTIAL AMINO ACID INDEX USING EGG PROTEINS AS THE STANDARD (Mitchell, 1954)

Amino Acid	*Amino Acids per 16.0 g. of N*		*Egg Ratios*	*Egg Ratios Corrected*	*Logarithm of Corrected Ratios*
	Whole Egg g.	*Lactalbumin* g.	%	%	
Histidine	2.4	2.3	96	96	1.98227
Lysine	7.0	10.5	150	100	2.00000
Phenylalanine	6.3	5.0	79		
Tyrosine	4.5	5.3	118		
Phenylalanine + Tyrosine	10.8	10.3	95	88	1.94448
Tryptophan	1.5	2.5	167	100	2.00000
Methionine	4.0	2.6	65		
Cystine	2.4	4.0	167		
Methionine + Cystine	6.4	6.6	103	78	1.89209
Threonine	4.3	6.0	140	100	2.00000
Leucine	9.2	12.1	132	100	2.00000
Isoleucine	7.7	7.5	97	97	1.98677
Valine	7.2	6.6	92	92	1.96379
Average log					1.97438
Average antilog				94*	

* Modified essential amino acid index of lactalbumin.

2. Growth Methods (*cf.* Allison, 1955)

Since a protein is a complex substance containing nine or more separate "growth factors" (the essential amino acids), blanket estimations of "protein quality" as in a biological test of the entire protein, do not advance the understanding of nutritional values of protein nearly as much as do studies which are based upon single amino acids as variables.

Growth of young rats was the chief criterion of amino acid adequacy in the elaboration of the lists of essential and nonessential amino acids (Rose, 1938; Womack, 1944). In the main, when just sufficient of the amino acid variable had been added for support of the maximal rate of gain attainable under experimental conditions, that amount was regarded as the minimal requirement. Most of these studies were conducted by feeding *ad libitum*.

As the deficiency of an essential amino acid in the diet becomes more severe, animals may begin to lose weight. The loss of weight, however, appears to be merely an extension of the growth processes into the zone where loss of protein tissue is greater than its formation (Almquist, 1947). An illustration of these facts is given in Figure 40. The growth curves are continuous downward toward a common negative rate of gain at complete deficiency of the amino acid. Most probably, this denotes continuations of the same fundamental processes that are in operation at positive growth rates, namely, 1) a tissue synthesis proportional to the supply of the amino acid, 2) a tissue degradation and loss relatively independent of the supply of the

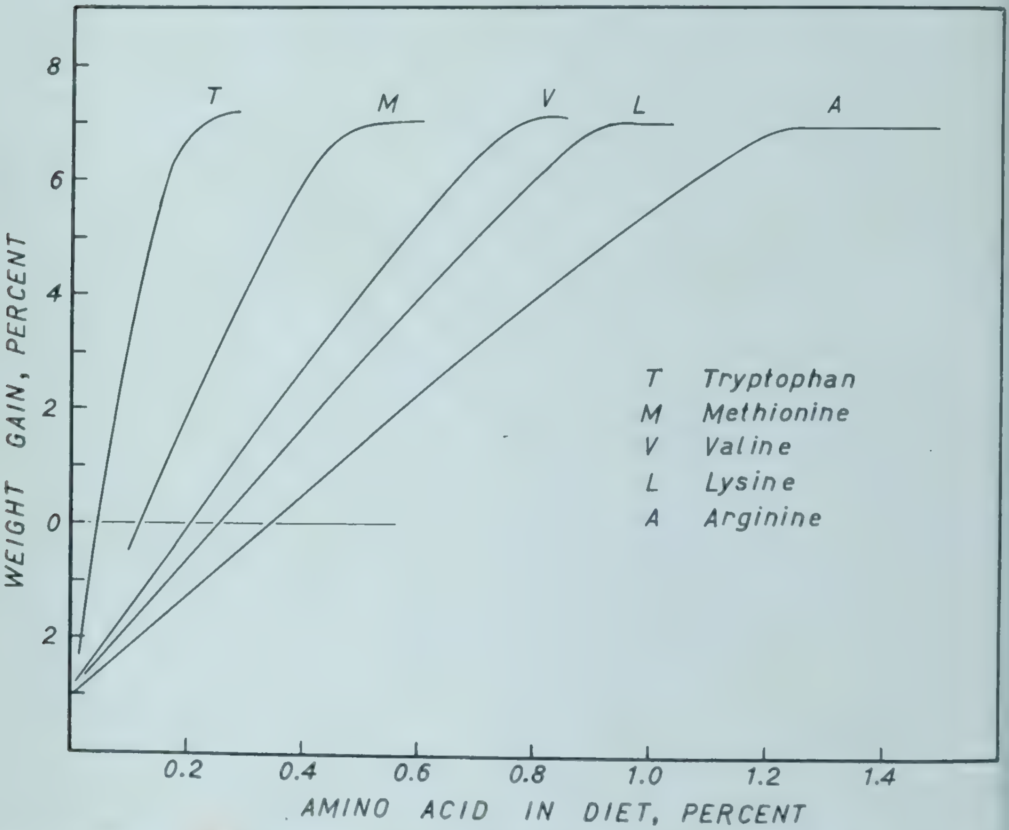

FIGURE 40. The relation of the daily rate of weight gain of chicks to the percentages of certain essential amino acids in the diet.

amino acid. Consistent growth responses to amino acid supply take place over the entire range from the lower limit of weight loss to the upper limit of weight gain, irrespective of the corresponding variations in food intake.

It was pointed out that the true zero point of growth should be represented by the point of maximum weight loss and not by the point of zero gain or loss. The latter may require as much as 30% of the total amino acid requirement for establishment of optimal gain. Where the lower limiting rate of gain is not attained under absence of an amino acid then that amino acid is being supplied from some source in small amounts or is being synthesized within the animal.

The Law of Diminishing Returns was applied to the analysis of growth data on amino acid requirements of chicks and poults (Almquist, 1952, 1953a, 1953b). The biological response to an increase in supply of some essential nutrient will often be found to follow a curve of constantly diminishing increment, i.e., a logarithmic curve. In such cases, plotting the data on a logarithmic intake basis will usually produce straight response lines up to the limit of response (*cf.* chapter IV). Such a limit may be set by physiological, genetic or other nonnutritional limitations. When the limit of maximal response is well defined, as by plateau in the data, the minimal requirement then is readily perceived from the intersection of the response and plateau lines. At a very low level of nutrient intake, the data may sometimes be seen to deviate from the principle, but, in this case, largely because reserve stores of the nutrient in the animal, synthesis within the animal, or small residual quantities in the basal diet are no longer negligible in respect to controlled intake of the nutrient. Furthermore, at the very low intake level animals may become ill or develop a pathology which indicates that they should not be considered normal.

A valuable feature of the logarithmic method is that full weight is given to the submaximal data. These are indispensable in establishing the location and slope of the response line. They facilitate the projection of the response line to a limit, as in the case of growth rate. All the data play a part in the estimation of requirement, not merely the data in the vicinity of the maximal response. Often, the latter are too meager for this purpose. Another good feature is that poor or inconsistent data are usually made quite apparent.

In Figure 41 are illustrated several examples of the application of this principle to amino acid requirement. The upper left portion of this figure was constructed from data on the arginine requirements of young chickens as affected by protein level at 15, 20 and 30% protein

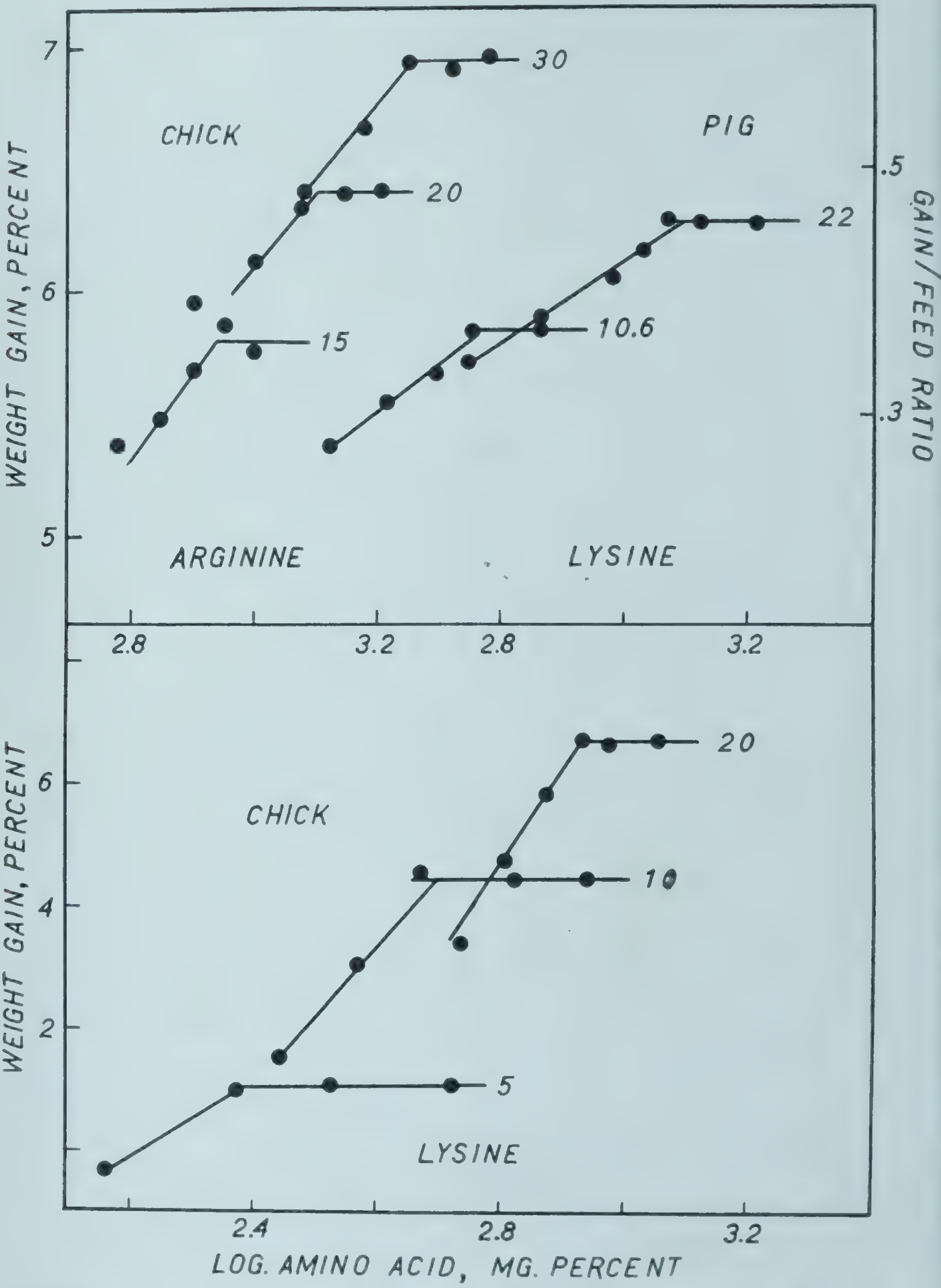

FIGURE 41. The relation of daily rate of weight gain of chicks, and the gain feed ratio of young pigs, to the logarithm of an amino acid level in the diet, at different levels of protein. Per cent protein in diets is indicated at the right.

in the diet (Almquist, 1950). Noteworthy is the evident linear relation of rate of growth to the logarithm of the total arginine level in the diet, over all protein levels. The relation reached a plateau of growth in each case as the protein level of the diet became the limiting factor.

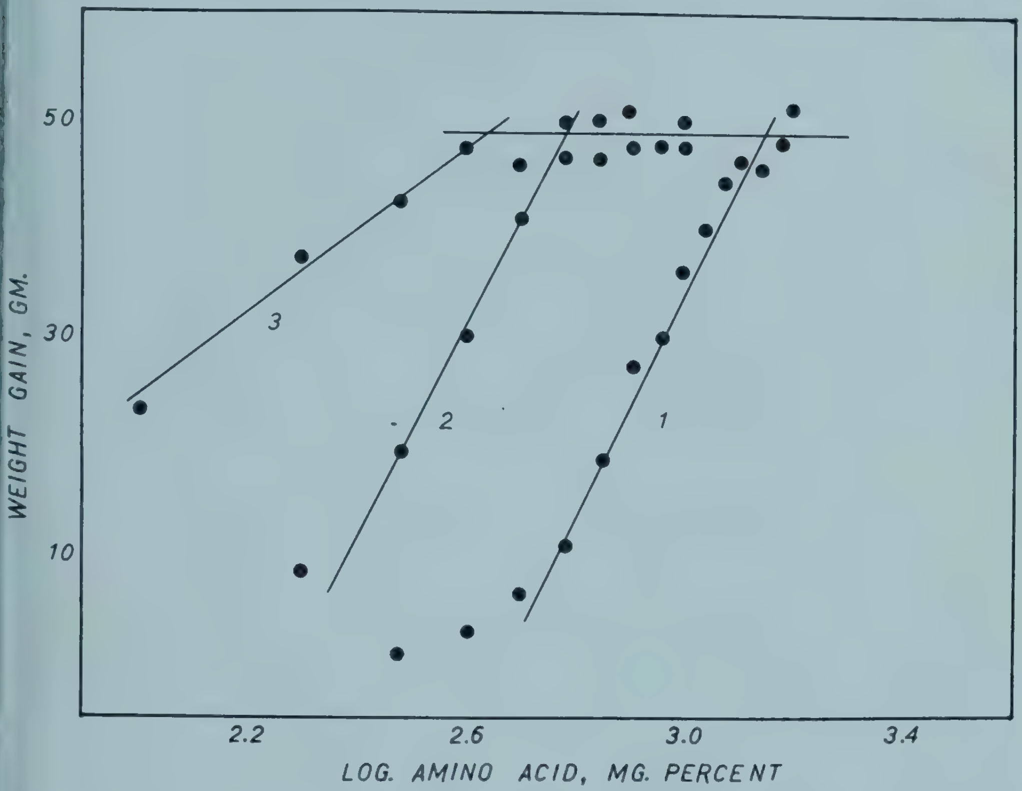

FIGURE 42. The relation of weight gain of rats to the logarithm of (1) the phenylalanine content of the diet with tyrosine absent, (2) the phenylalanine content of the diet with 1% L-tyrosine present, (3) the tyrosine content of the diet with 0.6% of L-phenylalanine present. From data of Armstrong (1955).

This fact shows that a deficiency of arginine gave the same net results as a deficiency of protein. The upper right portion was constructed from data of Brinegar (1950b) on the lysine requirements of the young pig at two dietary protein levels. The lower portion represents the results of Grau (1948) on the growth response of the chick to lysine at three dietary protein levels. The last two examples agree in essential features with the observations made concerning the arginine example.

In Figure 42 are presented in a similar way some data on the weight gains of young rats in relation to the intakes of phenylalanine and tyrosine as reported by Armstrong (1955). This figure illustrates several broad relations. (1) As previously mentioned, animals on very low intake levels appeared to deviate from the general course clearly followed by more normal animals on higher, although yet inadequate, intakes. (2) The growth response to phenylalanine in the absence of tyrosine was a measure of the need for phenylalanine to serve as a source of both these amino acids. When tyrosine was present in the diet, less phenylalanine was needed for a given growth rate but the proportion of the needs for phenylalanine in the two circumstances was constant, as shown by the parallel nature of the growth response lines. This indicates that a relatively constant proportion of the phenylalanine was converted to tyrosine when tyrosine was not provided in the diet. (3) When a minimum requirement of phenylalanine was provided, tyrosine additions improved growth rate, but along a response line of different slope, because the proportion of the phenylalanine which was necessarily converted to tyrosine was steadily diminished as the tyrosine intake was increased. (4) The total phenylalanine requirement, indicated at the junction of the response

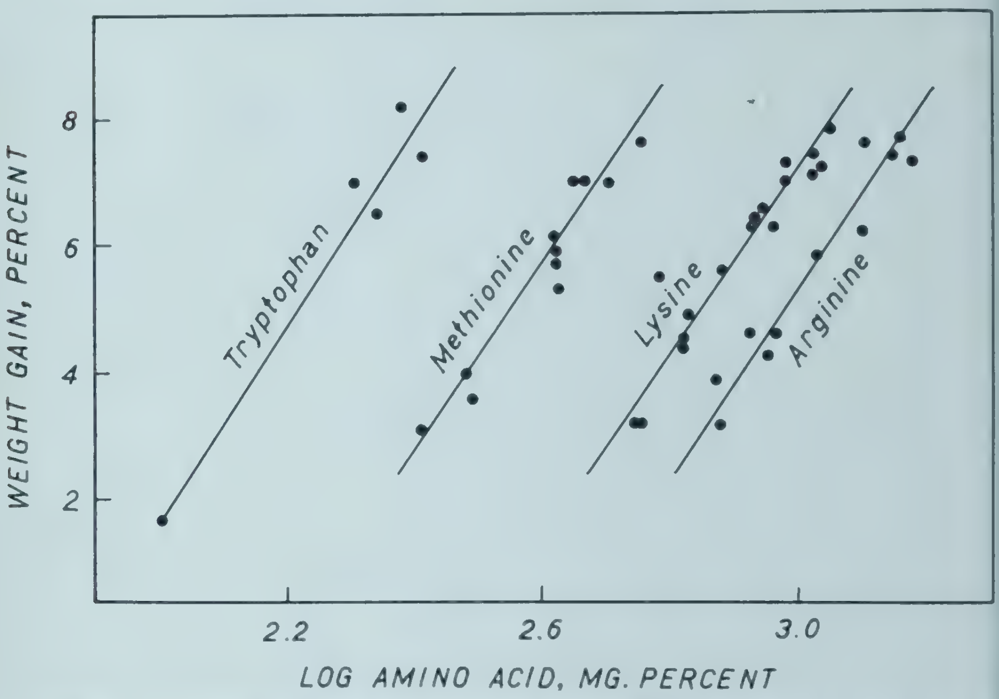

FIGURE 43. The relation of the daily rate of weight gain of chicks to the logarithms of the dietary contents of several amino acids.

and plateau lines, was 1.32% (i.e., antilog of 3.121), the amount needed in the presence of excess tyrosine was 0.60% (i.e., antilog of 2.778) and the minimum amount of tyrosine needed in the presence of 0.60% phenylalanine was 0.42%. Therefore, it appears that about 0.72% phenylalanine was needed to manufacture 0.42% tyrosine. The possible yield of tyrosine from 0.72% phenylalanine was 0.79%. Evidently, as pointed out by Armstrong, the conversion of phenylalanine to tyrosine was about only 50% efficient.

The sum of the needs for these two amino acids is clearly less than that for phenylalanine as the single source of them, and tyrosine has more than an equivalent "sparing" action on its precursor.

That requirements of essential amino acids for growth remain in similar proportions over a wide range of growth is further demonstrated in Figure 43, which illustrates the relation of chick growth rate to the dietary levels of tryptophan, methionine, lysine and arginine. The parallelism of the response lines is evidence of constant ratios of requirements (Almquist, 1954c).

3. Protein Efficiency and Regulated Nitrogen Intake Methods

Because of obvious depression in appetite and voluntary food intake of amino acid-deficient animals some investigators have felt that data obtained under such conditions could not be strictly quantitative. Refinements of method were proposed, including the calculation of ratio of weight gain to actual protein intake (efficiency ratio), the limitation of feed intake to similar quantities (paired feeding) and even forced feeding.

Hegsted (1947) compared gain in weight of rats with protein efficiency using 23 different proteins in diets of 12% protein content. He found a very high correlation between gain and protein efficiency and concluded that proteins may be classified relative to each other with equal accuracy by either gain in weight or efficiency. Sherwood (1953) compared *ad libitum*, adjusted protein, constant protein and paired feeding methods on young rats. The methods ranked in the listed order of descending sensitivity and that there was no advantage in protein efficiencies over simple gain in body weight was also noted. While these reports deal with over-all values of whole proteins, it is probable that the conclusions apply equally well to conditions under which specific amino acids can be varied independently.

Grau (1947) used amino acid mixtures in studies of phenylalanine and tyrosine utilization by the chick and concluded that rate of growth was the most important criterion of dietary adequacy in respect to amino acid supply. In similar investigations with rats, Steffee (1950) found a correlation between weight gain and gain in carcass protein of 0.96.

Rat growth and efficiency of protein utilization have been used in recent studies on amino acid supplementation of wheat protein. Rosenberg (1954) noted improvement in both measurements following the addition of lysine, but none with further additions of threonine and valine. On the other hand, Sure (1952, 1954) reported favorable effects on rat growth and protein efficiency from lysine, and also threonine and valine additions. Pecora (1951) reported that lysine and threonine are the limiting essential amino acids in white rice.

4. Nitrogen Balance Methods

The determination of biological values of proteins was developed to a reproducible method by Mitchell (1945). The method was based upon quantitative accounting for nitrogen intake and excretion under a paired-feeding procedure. For a further discussion of the factors influencing the significance of the "biological value" the reader is referred to reviews by Mitchell (1950), Frost (1950) and Almquist (1951a).

The relative rating of a protein based on protein efficiency, biological value and nitrogen balance varies with the total level of protein fed, the presence of other kinds of protein in the diet, substances which affect digestion and the adequacy of all other growth and health factors, especially the caloric intake (*cf.* Rose, 1954a). The same protein may be different in its value for growth as compared to maintenance.

Perhaps no subject in nutrition has had so much study, effort and

TABLE XIV

AMINO ACID REQUIREMENTS OF RATS
(Tryptophan as unity)

Amino Acid	*Adult-Total Repletion*	*Adult-Maintenance Only*	*Adult-Repletion Only*	*Young Rat Growth*
Tryptophan	1.0	1.0	1.0	1.0
Histidine	1.5	1.1	1.8	2.0
Lysine	3.7	2.0	4.8	5.0
Leucine	5.2	2.8	6.6	4.0
Isoleucine	4.3	5.4	3.7	2.5
Phenylalanine	3.3	2.2	3.8	3.5
Methionine	2.8	3.0	2.6	2.0
Threonine	3.2	2.8	3.2	2.5
Valine	3.6	3.7	3.6	3.5

discussion devoted to it. Yet progress was limited until the science of nutrition had developed, in respect to all other nutrients and the identification of amino acids, so that diets could be formulated which would permit studies of the roles of the individual amino acids.

Benditt (1950), using a diet containing an amino acid mixture in place of protein, estimated the requirement of the well-nourished adult male rat for nine essential amino acids to establish nitrogen balance. His results have been recalculated and are the basis for the maintenance column in Table XIV.

Frost (1950) has discussed nitrogen balance methods critically and has pointed out that maintenance of nitrogen balance alone is not a guarantee of protein or amino acid adequacy. The well-nourished animal requires more nitrogen from a given source to maintain equilibrium than does the depleted or standardized animal.

5. Protein Repletion Methods

Regeneration of liver protein of protein-depleted rats may be used to evaluate the quality of dietary protein (Campbell, 1948). This method requires more chemical analyses, and although shorter, appears to possess little or no advantage over simpler growth tests made under the same controlled conditions.

Restoration of weight of adult rats depleted of protein for a week or more and then fed a protein in the diet has been used extensively. The method has an advantage in that the same rats can be used several times. The amount of protein which will restore protein-depleted rats to maintenance of weight has been proposed as an index of quality (Tomarelli, 1947). The tested proteins were compared to a standard protein such as casein. Plasma protein regeneration may also be measured. An interesting variation of method has been employed by Arnold (1952) in which amino acid preparations or protein hydrolyzates suitable for intravenous administration were given intraperitoneally to depleted adult rats and the nitrogen balance estimated.

Steffee (1950) ascertained the amino acid requirements for repletion of adult male rats which had been depleted for several months on a ration low in protein but adequate in other respects. The rats lost 24 to 33% of their weight. They were given 15 grams daily of a repletion ration containing an amino acid mixture in which any one amino acid could be varied. The estimated requirements for maintenance and for repletion calculated in terms of tryptophan as a standard are given in Table XIV (*cf.*, Kandatsu, 1951).

It is of interest that the calculated values for adult repletion only agree best with the estimated requirements of the young growing rat. Since most demonstrated requirements show that the tryptophan requirement is 1.0% of the crude protein (or equivalent amino acid) intake these values may be read directly as grams per 16 grams of nitrogen in the diet. They are, of course, not to be accepted as final since further work may well bring revisions.

As an expression of requirements, grams per 16 grams of nitrogen does not indicate absolute requirements but rather serves to emphasize the important principle of proper proportions among the essential amino acids and the protein nitrogen level.

Nasset (1951) used nitrogen balance of depleted rats in a study of L- and DL-threonine. The latter was only one-half as active as the natural isomer. Threonine-deficient rats developed an acute edema of the esophagus. The requirement was reported as 20.1 mg. of threonine per day per kilogram of body weight to the three-quarters power. It is perhaps unfortunate that amino acid requirements have so often been related to time or to body weight, frequently without information as to level of nitrogen or protein intake or balance of other amino acids.

Guthneck (1953) conducted studies of the utilization of lysine from many food proteins by the adult protein-depleted male rat. For optimal growth the assay method used indicated a requirement of 0.45% lysine in a diet containing approximately 1.4% nitrogen, or 5.1 grams of lysine per 16 grams of nitrogen. Similar studies on methionine utilization from foods were reported by Schweigert (1954). It appears from a logarithmic analysis of their data that depleted adult rats require approximately 0.18% methionine in the diet while young growing rats require 0.26% in the diet. The diet probably contained the equivalent of 1.9% nitrogen; hence, the requirements may also be estimated as 1.5 and 2.2 grams of methionine per 16 grams of nitrogen.

Frost (1954) found that whole blood was seriously inadequate for repletion of adult rats until fortified with isoleucine and methionine. He emphasized the fact that whole blood or plasma given for shock or blood replacement could not also be considered a substitute for good-quality food protein. The inadequacy of blood proteins in isoleucine was pointed out by Block and Bolling in the first edition of this book (1945).

6. Analysis of the Carcass for Amino Acids

Comparison of carcass and diet composition with respect to amino acids is another way to estimate amino acid utilization and good dietary amino acid balance, if it is assumed that the proportions in the carcass are indicative of best proportions to be ingested. Most amino acid balances arrived at this way show relatively low recovery of amino acids, as might be expected (Grau, 1947). It has repeatedly been observed, however, that the proportions of certain amino acids in specific tissues were independent of the proportions in the diet (Evans, 1950; *contra* Kandatsu, 1951; Block and Bolling, 1951). These findings were reaffirmed by Price (1953) who analyzed two diets and the chicks reared upon these diets for 10 essential amino acids. Williams (1954) has reported the amino acid contents of whole carcasses of the rat, chick and pig, at different stages of growth, and has calculated amino acid requirements to correspond (*cf.* Block and Bolling, 1951; Kavanau, 1954a).

TABLE XV

COMPARISON OF CARCASS ANALYSIS WITH DIETARY REQUIREMENTS OF CHICKS
(Grams per 16 grams of nitrogen)

Amino Acid	*Carcass Composition*			*Dietary Requirement*
Tryptophan	1.0	0.8		1.0
Arginine	6.6	6.7		6.0
Histidine	1.8	2.0		1.5
Lysine	6.6	7.5		4.5
Leucine	7.2	6.6		7.0
Isoleucine	4.3	4.1		3.0
Phenylalanine	4.2	4.0	4.8	4.5
Methionine	2.0	1.8		2.3
Threonine	4.4	4.0		3.0
Valine	5.0	6.7		4.0
Tyrosine	—	2.5	2.9	—
Cystine	—	1.8		—
Glycine	—	10.1		7.5
Glutamic acid	—	13.3		—
References	Price (1953)	Williams (1954)	Grau (1947)	Almquist (1952)

A study of the reported distribution of amino acids of the chick, rat and pig shows that the correlation of carcass* analysis with present estimations of requirement is good (see Table XV and XVI). In general, the proportion of arginine, lysine, leucine, isoleucine, threonine and valine are higher in carcass protein than in diet protein; phenylalanine shows close agreement throughout, and methionine is uniformly slightly lower in carcass protein than in diet protein. In view of the known facts that some amino acids serve multiple purposes, aside from tissue synthesis, that some are irreversibly wasted metabolically while others can be regenerated or recovered, and that some are needed more intensively in certain tissues, it is not reasonable to expect that the correlation of carcass and diet composition should be very high. Carcass analysis may direct but not replace actual feeding experiments to measure amino acid requirements.

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* Widdowson (1951) reported the following composition of the human body: arginine, 6.6; histidine, 2.4; lysine, 7.1; tyrosine, 2.6; and glutamic acid, 12.6 grams per 16.0 grams of nitrogen.

TABLE XVI

COMPARISON OF CARCASS ANALYSIS OF RATS AND PIGS WITH DIETARY REQUIREMENTS

(Grams per 16 grams of nitrogen)

Amino Acid	*Rat*		*Young Pig*	
	Carcass Composition	*Dietary Requirement*	*Carcass Composition*	*Dietary Requirement*
Tryptophan	0.8	1.0	0.7	1.0
Arginine	5.9	1.0	7.1	1.5
Histidine	2.2	2.0	2.7	2.0*
Lysine	7.6	5.0	8.6	5.5
Leucine	6.5	4.0	7.1	6.0
Isoleucine	3.5	2.5	3.8	3.0
Phenylalanine	3.7	3.5	3.8	3.5*
Methionine	1.7	2.0	1.8	2.0
Threonine	3.9	2.5	3.8	3.0
Valine	5.5	3.5	6.0	3.0
Tyrosine	2.9	—	2.6	—
Cystine	1.5	—	1.0	—
Glycine	—	0.0	—	—
References	Williams (1954)	Womack (1944) Schweigert (1954) Wretlind (1950)	Williams (1954)	Almquist (1954b) Mertz (1954) Pfander (1955)

* A maximal estimate.

7. Determination of Minimum Amino Acid Intakes in Proteins of Adequate Diets for Nitrogen Balance

Mitchell (1950) presented estimates of rat amino acid requirements derived from diet composition, by calculating satisfactory minimal amounts. True minima may not necessarily be attained by this method if none of the diets examined approached an actual minimum requirement of a certain amino acid. However, the comparison is quite close.

TABLE XVII

MAINTENANCE AMINO ACID REQUIREMENTS OF ADULT RATS
(Tryptophan as unity)

Amino Acid	*Calculated from Diets*	*Directly Determined*
Tryptophan	1.0	1.0
Arginine	3.9	0.0
Histidine	1.4	1.1
Lysine	2.0	2.0
Leucine	6.1	2.8
Isoleucine	3.7	5.4
Threonine	2.2	2.8
Valine	4.2	3.7
Phenylalanine	—	2.2
Phenylalanine + Tyrosine	7.2	—
Methionine	—	3.0
Methionine + Cystine	3.4	—
References	Mitchell (1950)	Table XIV

in most respects, to rat maintenance requirements estimated by direct experiment (see Table XVII).

It is, of course, well known that the adult rat does not require arginine. The directly determined figures for isoleucine and threonine may be too high, for both Mitchell (1950) and Womack (1953) have indicated much lower requirements. The methionine estimates may also be too high, since Womack (1953) has reported that only about 30% of the total sulfur amino acid need be in the form of methionine.

TABLE XVIII

MAINTENANCE AMINO ACID REQUIREMENTS OF ADULT MAN
(Tryptophan as unity)

Amino Acid	*Calculated from Diets*	*Directly Determined*
Tryptophan	1.0	1.0
Arginine	3.7	0
Histidine	1.7	0
Lysine	3.1	3.2
Leucine	5.8	4.4
Isoleucine	3.5	2.8
Threonine	1.8	2.0
Valine	3.7	3.2
Phenylalanine + Tyrosine	7.6	4.4
Methionine + Cystine	3.3	4.4
Body Weight	70 kg.	70 kg.
Total Nitrogen Intake	—	10 g.
References	Mitchell (1950)	Rose (1949-1955)

Mitchell (1950) calculated the amino acid requirements of adult man. These calculations may be compared with the direct determinations made by Rose and associates (1949-1955) on young human male subjects (Table XVIII). As in the example of the rat data, the agreement of the calculated and directly determined values is to be regarded as quite good.

Many questions and imponderable factors enter into these determinations and it is not the present purpose to enter into an inclusive discussion of them. Instead, the reader is referred to the reviews by Mitchell (1950), Frost (1950), Rose (1949) and Almquist (1951a, 1951b).

8. Free Amino Acids in the Blood

Free amino acids in the blood and amino acids in the diet of chicks have been reported by Charkey (1950, 1953) and Richardson (1953). These reports have been reviewed (Almquist, 1954a). Typical results are given in Table XIX. Three diets of quite different amino acid patterns are listed together with the corresponding free amino acid content of chick blood and the estimated dietary amino acid requirement of the chick.

Without exception, the order of abundance of any free amino acid in the blood was in agreement with the order in diet protein. In the case of a deficiency of an amino acid in the diet, the same amino acid in blood dropped to a comparatively low level. All diets were, evi-

TABLE XIX

FREE AMINO ACIDS IN CHICK BLOOD IN RELATION TO DIET AND REQUIREMENTS

Amino Acid	*Present in Blood**			*Present in Diet Protein†*			*Requirement in Diet Protein†*
	SS Diet mg. %	*SBM Diet mg. %*	*PM Diet mg. %*	*SS Diet*	*SBM Diet*	*PM Diet*	
Arginine	4.9	9.4	9.8	5.33	6.10	7.83	6.0
Lysine	11.0	6.9	1.5	6.56	5.27	2.96	4.5
Methionine	4.8	3.4	2.9	2.06	1.45	0.99	2.3
Cystine	—	—	—	0.82	1.96	1.68	2.0
Tryptophan	6.2	7.6	6.4	1.13	1.20	1.15	1.0
Valine	19.1	17.6	13.6	5.95	5.38	4.90	3.0

* Composition of Diets:

SS Diet: milo, 22; soybean meal, 15; casein, 14.5, gelatin, 3.0; nonprotein constituents and vitamins to 100 (Richardson, 1953a).

SBM Diet: milo, 66.5; soybean meal, 30; nonprotein constituents and vitamins to 100 (Richardson, 1953a).

PM Diet: milo, 64.5; peanut meal, 32; nonprotein constituents and vitamins to 100 (Richardson, 1953b).

† Calculated as grams per 16 grams of nitrogen.

dently, deficient in total sulfur amino acids, and blood methionine values were corresponding low. Tryptophan was supplied in a nearly equivalent amount and in slight excess throughout, causing nearly equivalent values in blood. In all cases, valine was provided in large excess and was the most abundant free amino acid in the blood.

It may be observed that when the diet supply was closely comparable to known requirement there was little tendency to a relative accumulation of the amino acid in blood. On the other hand, a surplus or a deficiency of an amino acid in the diet was reflected in exaggerated degree in blood. This observation would be expected if the withdrawal of amino acids from blood followed a certain pattern, independent of diet. It is evident that free amino acids in blood should be considered not only in respect to those in diet, but also in respect to requirement of the animal.*

The close relation between free amino acids in blood and amino acids bound in food protein requires a process in which amino acids are liberated during proteolysis in proportions similar to those in the protein and then are absorbed comparatively rapidly, in approximately those same proportions, into the circulation. The proportionality of requirements was not appreciably altered whether amino acids were provided as intact protein or as mixtures of crystalline amino acids (Almquist, 1947).

These observations suggest another method for evaluating amino acid requirements, and one which perhaps might be regarded as the most direct method of all. Since it is quite evident that relative surpluses or deficiencies of amino acids in diet tend to be magnified in the free amino acids in the blood, it may well be possible to employ blood amino acid content as a sensitive index to amino acid adequacy in the diet. It could be assumed, if the pattern of free amino acids in the blood remained closely similar to that ingested, that the latter had been brought to a balance with the needs of the animal. With present micro-methods it should be possible to conduct the analyses on relatively small blood samples and to use the same animals repeatedly.

The increases in concentration of free amino acids in the blood of dogs were found to be proportional to the amounts fed in the protein.

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* Eagle (1955) reported the specific amino acid requirements of a mammalian cell in tissue culture to be the following: arginine, 0.05; histidine, 0.01; lysine, 0.05; tyrosine, 0.02-0.05; tryptophan, 0.005; phenylalanine, 0.02; cystine, 0.01; methionine, 0.02-0.05; serine, 0.0; threonine, 0.05; leucine, 0.05; isoleucine, 0.1-0.2; valine, 0.05; glycine, 0.0; alanine, 0.0; glutamic acid, 0.0; aspartic acid, 0.0; proline, 0.0 millimoles.

The amino acids were present in much larger amounts in the portal vein as compared to the radial vein (Denton, 1953). Denton (1954a) fed casein, zein and beef protein to dogs and observed that the concentration of free amino acids in portal plasma soon increased, approximately in the proportions fed, thus indicating similar availabilities of the amino acids. When an amino acid mixture was fed (Denton, 1954b), all amino acids were rapidly absorbed with the exception of phenylalanine and, possibly, of leucine.

TABLE XX

AVERAGE VALUES FOR CERTAIN FREE AMINO ACIDS IN HUMAN BLOOD

(Charkey, 1955)

Amino Acid	*Full Food mg. %*	*Fasting mg. %*	*Return-to-Food mg. %*
Lysine	4.65	4.00	4.00
Threonine	2.07	1.32	1.49
Methionine	0.25	0.23	0.39
Arginine	2.12	1.76	1.43
Leucine	3.88	6.03	4.69
Tryptophan	0.92	0.65	0.87
Valine	5.81	7.62	5.63

Steele (1950) reported amino acids in protein-free filtrates of human plasma. The subjects were given essentially the same diets in amounts to provide different levels of protein. The lowest level of protein was 25 grams daily which is sufficient for maintenance. Since the proteins consumed were the same at all levels it is not surprising that the blood free amino acids were present in similar amounts regardless of protein level consumed. Nonessential amino acids were found most abundantly in the urine. There was little or no relation between blood free amino acids and amino acids in urine.

Charkey (1955) has reported certain amino acids in the blood of humans during full food, fasting and return-to-food periods. The subjects were not maintained on a controlled diet. The average values for 6 adult individuals are given in Table XX. There was a large range in variation among the individual blood free amino acid values, possibly due for the most part to a free selection of diet. It is evident, therefore, that there can be no "standard" values for blood free amino acids.*

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* It has been reported that there is little correlation between the patterns of the free amino acids in the tissues and those of the corresponding whole tissues (Solomon, 1951).

A good deal of attention has been directed to the excretion of amino acids. This work has been of great value from a metabolic standpoint but has little or no quantitative bearing on amino acid requirements (*cf.* Eades, 1954).

9. Microorganisms for Protein and Amino Acid Assays

Microbiological methods for amino acid determinations are well established. If some organism could be found whose amino acid needs were similar to those of higher animals, such an organism might be used in rapid and inexpensive estimation of protein value and amino acid requirements. Pilcher (1954) described such a method based upon the protozoa *Tetrahymena pyriformis* W. The growth response of this organism to partially purified proteins indicated that the proteins ranked in the same order as found in bioassays with higher animals. The amino acid composition of the protozoa was similar to that of vertebrates. Likewise the insects *Tribolium confusum* and *Blattella germanica* may be of value in bioassay of proteins (Lemonde, 1951; Fraenkel, 1954; Hilchey, 1953). In order to establish firmly the usefulness of such organisms in testing proteins or amino acid sources for their nutritive value for higher animals, a great deal of simultaneous testing must be done with all assay animals. It seems possible, however, that the microorganism method might prove useful in quality control during the manufacture and processing of protein foods.

OTHER ASPECTS OF AMINO ACID REQUIREMENTS

1. Utilization of *D*-Amino Acids

The question of the nutritional value of the unnatural isomers of the amino acids does not constitute a practical problem. Only two synthetic amino acids are, at present, used in human and in animal feeding. It happens that one of these, DL-methionine, is, for all practical purposes, fully as active as the natural form. Another amino acid which may see increasing use, DL-lysine, is only half as active as the L-form. It may be that the latter will be made available for use in animal diets, as well as for humans (*cf.* Brockington, 1950), if the resolution of the DL-lysine can be achieved economically.

No phase of amino acid requirement is more complicated than the question of the values of the D-isomers. The subject is an important one in application to research with amino acid mixtures. The reader is referred to the excellent papers on this subject by Berg (1953) and by Phillips (1954) and discussions in the reviews previously cited (Frost, 1950; Almquist, 1951a, 1951b).

Rats fed suboptimal amounts of all the essential amino acids simultaneously grew somewhat better when the poorly invertible group (valine, leucine, isoleucine, lysine* and threonine*) was supplied as DL-forms at twice the L level fed. It appears that the D-isomers provided some amino nitrogen to facilitate the synthesis of the nonessential amino acids. This effect was more readily manifested by glycine or ammonium compounds (*cf.* Frost, 1950).

The group of amino acids which are readily invertible did not fully replace their pure L-form when fed at the same level in the DL-form. Even less growth was obtained when this group was fed as the D-isomers. At lower levels of nitrogen intake many DL-amino acids become more nearly equivalent to the L-forms.

It appears that a limit is imposed upon the over-all capacity of the animal to invert heavy doses of D-amino acids, even of those whose inversions are readily achieved when fed singly (Wretlind, 1952a, 1952b; Phillips, 1954).

Almquist (1951b) observed "in those cases where an indispensable amino acid fed in DL-form is clearly depressing to growth it is also true that the D-component is utilizable to an appreciable extent for growth. In those examples in which the DL-amino acid is not inhibitory, the D-isomer is not appreciably used for growth. The significance and constancy of this relation remains to be examined."

2. Relation of Amino Acid Requirement to Protein Level in the Diet

The nearly linear relations of growth responses to amino acid levels in the suboptimal zone indicates relatively fixed proportions of amino acids requirements (Almquist 1947, 1949). Grau (1948) demonstrated that for best growth, the chick required an intake of lysine that was in proportion to the protein intake. This finding was confirmed for lysine and extended to include total sulfur amino acids by Grau (1950).

The methionine requirement of chicks was found to be approximately 2.5% of the protein at 20 and 30% protein levels (Almquist, 1949). The arginine requirement of the chick was found to be approximately 6% of the protein in diets of 15, 20 and 30% protein (Almquist, 1950, see also Figure 40). Kratzer (1950) observed that the lysine requirement of the poult for maximal growth was related to dietary protein level. Also, the requirement for prevention of a white barring of feathers, a specific manifestation of lysine deficiency, was closely related to protein level.

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* These two amino acids cannot be inverted due to the instability of their keto analogues.

The optimal level of lysine in diets for young pigs was found to be approximately 5.5% of the dietary protein at levels of 10.6 and 22% protein (Brinegar, 1950). This author stated that the amino acids requirements would be best expressed as percentages of the dietary protein, as suggested in the first edition of this book (Block and Bolling, 1945; *cf.* Block, 1944b).

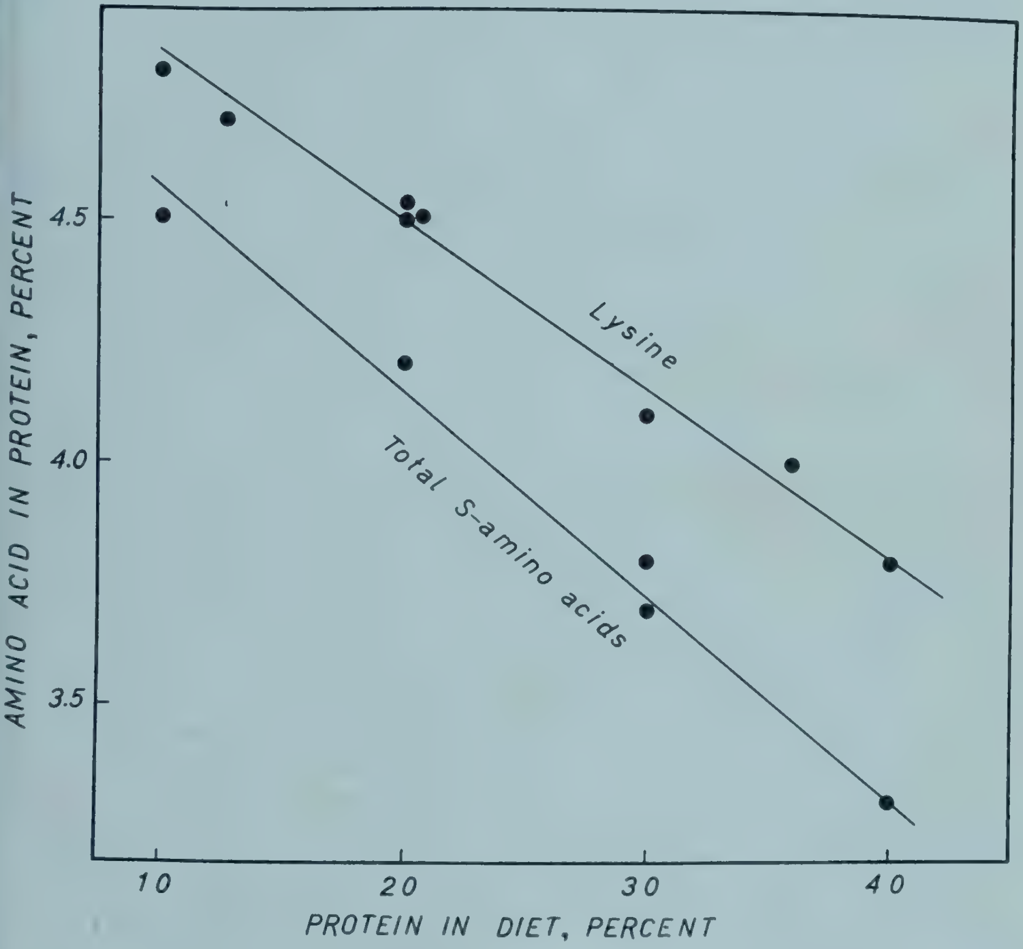

FIGURE 44. Relation of the optimal percentage of lysine and of total S-amino acids in the protein to the level of protein in the diets of chicks.

There is some evidence that, as the gross protein level is raised, the percentage of essential amino acid required in the protein may decrease. Almquist (1952) collected the evidence on this question, as presented in Figure 44. This shows that the lysine and total sulfur-amino acids required in the protein of chick diets decreased as the protein level was raised. It seems quite probable that the same relation would apply to the other essential amino acids, but this question remains to be demonstrated experimentally.

It is well known that protein efficiencies tend to improve at lower levels of protein intake. Since the fraction of the protein intake which is metabolized for energy must increase with the level fed and such energy utilization may be directed especially toward the nonessential amino acids, or does not require the essential amino acids, it may be that correspondingly lower but *properly proportioned* levels of essential amino acids in the protein will suffice at higher protein intake.

It has been demonstrated adequately that amino acid requirements vary almost directly with the protein intake. In other words, each essential amino acid must remain in balance with the dietary protein and other amino acids for most effective utilization. Requirements,[*] therefore, should be considered in reference to the protein level of the diet or total protein and amino acid nitrogen intake. This principle, which is almost self-evident, is still widely disregarded.

3. Relations of Vitamins to Amino Acid Requirements

It is well known that methionine can furnish the methyl groups for the biosynthesis of the essential nutrient, choline. This transfer is reversible. A choline deficiency can be overcome, in the rat, if sufficient extra methionine is provided; also in the chick if, in addition, mono- or dimethyl aminoethanol is furnished in the diet. Conversely, if sufficient choline is present only a minimum amount of methionine is needed, primarily for tissue protein synthesis, although a certain degree of demethylation of methionine probably always takes place as an automatic reaction.

Homocysteine, which is demethylated methionine, is utilized efficiently as a substitute for methionine only when a methylating substance such as choline is also made available (*cf.* Jukes, 1947). Methionine requirements should be estimated only with diets which are not deficient in choline.

Deficiency of niacin or pyridoxine in the diet seems to increase the toxic effects of certain amino acids fed in surplus amounts and this may indicate a general role of these vitamins in amino acid metabolism (Anderson, 1951). More specifically, it is known that tryptophan may be used as a precursor of niacin, hence a good deal of interest has been shown this relation. Niacin deficiency is hard to produce except when the diets are deficient or marginal in tryptophan. A secondary amino acid deficiency, which further impairs utilization of the protein for growth, may release tryptophan for niacin synthesis and actually improve the observed growth somewhat if the niacin deficiency is preponderant (Henderson, 1953; Koeppe, 1955). From the standpoint of amino acid requirements it seems that those determined during niacin deficiency are subject to reservations.

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[*] Luckey (1954a) has suggested a single diet for all living organisms. This suggestion indicates cognizance of the fact that protoplasm does not differ greatly in its composition.

Much has been said concerning the direct tryptophan-sparing action of niacin. The amount of tryptophan theoretically required to furnish 2.5 mg. of niacin per 100 g. of diet is 4.2 mg. A marginal dietary level of tryptophan is 100 mg. Therefore, the production of all niacin from tryptophan would hardly consume more than 5% of the total tryptophan needed. However, in order to synthesize niacin, there is much wastage of the amino acid, consequently the drain on tryptophan is considerable.

Hsu (1952) reviewed the subject of vitamin B_{12} and growth inhibitions by excess of amino acids. She reported that certain amino acids affected the vitamin requirement. The growth inhibition by the protein, zein, was attributed to its high content of leucine.

Sanford (1954) extended the work on tyrosine tolerance and vitamin C. Chicks fed a diet high in tyrosine showed a growth depression which was relieved when vitamin C was incorporated into the diet. In the absence of vitamin C, metabolites of tyrosine such as homogenetisic acid and keto-acids were excreted, but the feeding of vitamin C reduced this abnormality.

There are many reports of interactions between vitamin and amino acid supplementations of diets. Often these are interpreted as a metabolic interrelation between the nutrients in question. There is nothing exceptional in the fact that under a dual deficiency in the diet, animals may show a growth response to the correction of one deficiency or the other. However, this does not prove a metabolic interrelation except generally via growth rate or well-being. Only where the effect of the vitamin has been shown on some distinct phase of the intermediary metabolism of one or more amino acids is it justifiable to conclude that the vitamin is directly concerned in protein or amino acid utilization.

4. Relation of Amino Acid Requirement to Size, Age and Variety

Very young fowls are required to divert a large proportion of their sulfur amino acid intake to the growth of feathers which may contain as much as 10% cystine. The sulfur amino acid requirements may be more intense in smaller fowls which have relatively more surface area per unit of body weight. The available data on sulfur amino acid requirements indicate the relative needs of young fowls as given in Table XXI.

This comparison of relative concentration of sulfur amino acids indicates that smaller fowls do require higher proportions (Almquist, 1952). Mitchell (1950) has discussed similar differences in sulfur amino acid requirements of rats and humans and has related these requirements to differences in intensity of demand at different ages for formations of keratin proteins as compared to protoplasmic proteins.

TABLE XXI

APPROXIMATE SULFUR AMINO ACID REQUIREMENTS OF YOUNG FOWLS
(Grams per 16 grams of nitrogen)

Variety	*Methionine*	*Cystine*	*Total*
White leghorns	2.5	2.0	4.5
Heavy meat chickens	2.3	1.7	4.0
Turkeys	2.0	1.3	3.3

Hegsted (1941) observed that a rapidly feathering breed of chickens (Leghorn) showed distinct growth response to arginine and glycine supplements, while a slowly feathering breed (Plymouth Rock) showed little response.

Another requirement which may be related to speed of growth is that for lysine. This amino acid is concentrated to greatest degree in muscle and organ tissues and is relatively low in keratin proteins. It may be noted that the lysine requirement of the young chick is 4.5 grams per 16 grams of nitrogen at normal protein levels, while that of the poult is 5.4 grams, and of the young pig 5.5 grams.

The methionine and trytophan requirements appear to decrease with age of chicks of the same breed (Lewis, 1951). Pepper (1955) estimated that the methionine requirement of the turkey after 8 weeks of age will drop to 1.5% of the protein. The results of Gartley (1950) and of Kratzer (1955) indicate that the lysine level required in the dietary protein of the poults is lowered with age of the bird.

The concentration of methionine, tryptophan, leucine and isoleucine required in the proteins of the hen's diet is approximately the same as for the young chick. The lysine requirement of the laying hen is relatively lower than that of the chick (see Table XXII). This may

TABLE XXII

REQUIREMENTS FOR ESSENTIAL AMINO ACIDS[a]
(Grams per 16 grams nitrogen)

	Honey Bee[b]	*Rat*		√ *Chicken*		*Turkey,*	*Pig,*	*Dog,*	*Human*			
	Young	*Young*	*Adult*	*Young*	*Adult?*	*Young*	*Young*	*Adult*[c]	*Infant*[f]	*Adult*[g]	*Adult*[h]	*Adult*
Tryptophan	1.0	1.0	1.0	1.0	1.0	0.93	1.0	1.1	1.0	1.0	0.7	1.0
Arginine	3.0	1.0	0.0	6.0		5.7	1.5	6.2	0.0			0.0
Histidine	1.5	2.0	1.1	1.5			2.0	2.2	0.0			0.0
Lysine	3.0	5.0	2.0	4.5	3.3	5.4	5.5	7.1	5.6	4.5	5.6	3.2
Leucine	4.5	4.0	2.8	7.0	6.7		6.0	8.8	14.0	4.7	5.4	4.4
Isoleucine	5.0	2.5	5.4	3.0	3.3	2.9	3.0	6.2	3.0	5.3	3.7	2.8
Methionine	1.5	2.0	3.0	2.3	1.9	1.9	2.0	7.1[d]	2.8	3.8[d]	1.5	0.9[i]
Phenylalanine	2.5	3.5	2.2	4.5[j]			3.5	9.2[e]	5.6	6.8	3.1	4.4[e]
Threonine	3.0	2.5	2.8	3.0			3.0		2.9	4.4	2.5	2.0
Valine	4.0	3.5	3.7	4.0			3.0	6.9	5.4	4.5	4.3	3.2
Glycine		0.0		7.5		3.6						
Protein level of diet in %		20.0	10.0	20.0	15.0	28.0	20.0	6.5				

[a] When the diet contains all other amino acids of significance for requirements, plus adequate levels of nonessential amino acids or available nitrogen.

[b] de Groot (1954).

[c] Allison (1951).

[d] Includes cystine.

[e] Includes quota for tyrosine replacement.

[f] Albanese (1950).

[g] Hegsted (1955), all-vegetable diet.

[h] Reynolds (1953 mean amino acid intake for nitrogen balance in women. Cystine was not determined.

[i] Estimated from Rose (1953).

[j] Fisher (1956) finds the L-phenylalanine requirement of the chick to be 2.5.

be related to the fact that proteins of the egg are lower in lysine than are those of chicken muscle tissues.

Estimations of amino acid requirements of the rat as shown in Table XIV, indicate that the adult, as compared to the young rat, needs relatively much less lysine and phenylalanine, but relatively more methionine and, perhaps, isoleucine.

5. Analogues and Antagonists of Amino Acids

Many of the alpha-hydroxy and alpha-keto analogues will serve as dietary replacements for amino acids. The only essential amino acids not so replaceable are lysine and threonine as well as the semi-essential, cystine. The hydroxy-analogue of methionine is being manufactured for use in animal mixed feed. It appears to be fully as active as the amino acid itself (Bird, 1952). Ethionine, the analogue in which the methyl group of methionine is replaced by an ethyl group, is well-known to be a potent antagonist apparently involving the methylation functions of the amino acid (Stekol, 1951).

A more surprising discovery, and one of more practical importance, is the antagonism to isoleucine manifested by leucine* (Harper, 1955). These essential amino acids differ only in that the carbon chain of the isoleucine is branched. An excess of dietary L-leucine was found to retard the growth of rats fed low-protein diets or diets deficient in isoleucine (*cf.* Part II, amino acid composition of blood proteins.) The growth retardation was overcome by the addition of more isoleucine. It seems possible that this antagonism might also be demonstrated in reverse. Harper (1955) has reviewed the subject of abnormal growth effects of amino acids and has provided an extensive list of references.

6. Availability and Utilization of Amino Acids From Foods

The availability of amino acids in roast beef, cottonseed flour, peanut flour and wheat was studied with rats (Kuiken, 1948). The measure of availability was the difference between amount of amino acid fed in the diet and the amino acid found in feces.† The latter was corrected for the amino acid found in feces, during a period when the animal was fed only a low level of defatted whole egg protein, which is considered to be completely available.

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* Valine and phenylalanine also impair leucine utilization (Benton *et al.*, Arch. Biochem. and Biophys. *60*, 147 and 156, (1956)).

† Chemical methods for the estimation of the availability of certain amino acids have been described by Carpenter (1955), Mauron (1955), Sheffner (1955), etc.

The ten essential amino acids measured were all highly available in roast beef, peanut flour and wheat (92 to 100% available) while in the cottonseed meal, the range was from 64.5 to 93.4%. It was suggested that the manufacturing procedure might have adversely affected the amino acids in the cottonseed meal.

The utilization of lysine from a number of foods was studied by means of protein-depleted adult rats fed a lysine-deficient diet plus the tested food at a level to provide a standard amount of lysine. Utilization was calculated on the basis of rates of gain (Guthneck, 1953). The percentage used ranged from 49 to 98 for nineteen different foods. Unheated soybean flakes gave the poorest lysine results. A tendency for lower utilization was noted from cooked and processed meats as compared to raw meats. Similarly, utilization of methionine in these foods was found to be from 48 to 83% (Schweigert, 1954a). The lowest value was found with the unheated soybean flakes, as in the case of lysine. The lower range of results with methionine suggested that appreciable amounts had been used for purposes other than tissue synthesis. No consistent effects of processing or cooking of meats were noted.

The degree to which an amino acid in a food appears to be utilized by the adult protein-depleted rat for tissue restoration does not necessarily measure full availability, but it does give some expression of factors or treatment which may modify availability. The general effects of proteolytic enzyme inhibitors and of overheating of proteins have been discussed elsewhere (Almquist, 1951b; 1953c).

For years, methods have been sought to make available the amino acids in the enzyme-resistant types of proteins such as hoof, horn, hair, and feathers. Meals from these materials are usually made by treatment with steam under pressure. Several reports have appeared describing feeding tests with these products. Wilder (1955) tested steam-processed feather meals in chick diets and obtained some indications that the keratin was metabolized. Slinger (1955), on the other hand, found that when hoof and hair were digested with steam under pressure, or ground to a very fine state, the keratins were not utilized for growth of chicks and had no beneficial effects on feed efficiency. A commercial feather meal was found to be highly insoluble and useless as a source of amino acids to a strain of lactobacillus (Block, unpublished).

At present, and except where there is specific evidence to the contrary, it seems permissible to assume that amino acids in most feeds and foods are fully available for practical purposes.[*]

PROVISION OF AMINO ACID REQUIREMENTS

The past 25 years or so have seen a dramatic change in the principles and practice of nutrition. This may be described as a transition from an ingredient basis of feeding to one of a nutrient basis. In other words, what took place was the abandonment of empirical feed formulas in favor of the consideration of specific requirements of amino acids, vitamins, minerals, etc.

TABLE XXIII

REQUIREMENTS FOR ESSENTIAL AMINO ACIDS[a]
(As % of diet)

	Rat		*Chicken*		*Turkey,*	*Pig,*	*Human*	
	Young	*Adult*	*Young*	*Adult*	*Young*	*Young*	*Infant*[b]	*Adult*[c]
Tryptophan	0.2	0.10	0.20	0.15	0.26	0.2	30	0.25
Arginine	0.2		1.20		1.60	0.3	0	
Histidine	0.4	0.11	0.30			0.4	0	
Lysine	1.0	0.20	0.90	0.50	1.50	1.1	170	0.80
Leucine	0.8	0.28	1.40	1.00		1.2	425	1.10
Isoleucine	0.5	0.54	0.60	0.50	0.80	0.6	90	0.70
Methionine	0.4	0.30	0.45	0.28	0.53	0.4	85	0.22[d]
Phenylalanine	0.7	0.22	0.90			0.7	169	1.10[e]
Threonine	0.5	0.28	0.60			0.6	87	0.50
Valine	0.7	0.37	0.80			0.6	161	0.80
Glycine	0.0		1.50		1.0			
Protein level as % of diet	20.0	10.0	20.0	15.0	28.0	20.		

[a] When the diet contains all other amino acids of significance for requirements plus adequate levels of nonessential amino acids or available nitrogen.
[b] Mg. per kilo of weight.
[c] Grams per day.
[d] Estimated from Rose (1953).
[e] Includes quota for tyrosine requirement.

The pioneer work done on the qualitative and quantitative needs of the rat for the amino acids has been and is being extended to cover farm animals and humans. The requirements as known to date for several monogastric species are listed in Table XXIII.

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[*] It is interesting to note that methionine is absorbed through the skin (Edwards, 1954).

A gap in the table indicates that the requirement has not yet been demonstrated or estimated.

1. Summary of Amino Acid Requirements

The values in Tables XXII and XXIII were chosen on the basis of data already cited and for the conditions that all other amino acids of nutritive importance, i.e., cystine, tyrosine and sufficient of the nonessential amino acids or other nitrogenous sources were provided. There is no completely satisfactory way to formulate such a table. If expressed as a per cent of the diet then the requirements must also be accompanied by the corresponding protein level. Often different protein levels are desirable and in such cases the listed requirements must be recalculated, in proportion to the protein levels.

It is quite difficult to compare estimates of amino acid requirements for different species if the list is not adjusted for the often wide differences in protein contents of the experimental diets employed. For example, the swine requirement data were obtained over a range of 13 to 25% protein. In Table XXII these have been adjusted to a given protein level. For most purposes this adjustment to a uniform level is permissable and does not magnify particularly any errors which may already exist. As previously mentioned, the proportionality may not keep exactly in step with protein level, but has a tendency to drift toward lower percentages of essential amino acids required in the protein at higher levels of protein.

Another way to express the data generally is in terms of grams of amino acid per 16 grams of dietary nitrogen. The 16 factor is, of course, related to the common factor (6.25) for calculating crude protein from nitrogen percentage. The 6.25 factor is known to be incorrect for several types of single sources of food proteins, yet for accounting of protein in mixed diets the 6.25 average factor is established and apparently will be retained. There seems no point in using one factor for a feed when fed singly and another for the same feed when fed in a mixture. The argument whether a factor of 5.7 or 6.5 should be used is rather academic and fruitless.

Expressing amino acid requirements on the basis of grams per 16 grams of nitrogen has the advantage of emphasizing uniformity and balance of requirements. For employment of this expression in calculations, the nitrogen content of the diet must be known.

In Tables XXII and XXIII the amino acids requirements have been compiled both ways. It happens that in nearly all cases tryptophan requirement is 1% of the dietary crude protein or 1 gram of tryptophan is needed per 16 grams of nitrogen. Therefore, these figures also express the ratio of amino acid requirements with tryptophan as unity.

Data for farm animals are based upon the reviews by Almquist (1952) for poultry, and (1954b) for swine, with added references as cited previously and below. Data for rats have been given in the text and those for humans are taken from the reports by Mitchell (1950), Rose (1949-1955), and Albanese (1950), *cf.* Baptist (1953).

2. Other Reports on Amino Acid Requirements

Data of Wretlind (1950) indicate that the young rat requires 2.0 grams of methionine per 16 grams of nitrogen in the diet.

Young (1953), in a study of unidentified factors has reinvestigated the arginine requirement of the chick fed a purified diet. The requirement observed with several variations of the diet was 1.4% in the presence of 23% protein or 6 grams of arginine per 16 grams of nitrogen which is in close agreement with earlier estimates. Baldini (1955) found that methionine requirement of the chick is related to energy content of the diet and that previous variable reports on this requirement could be reconciled on an energy basis. In a reinvestigation of the question of glycine requirement of young chicks, Wixom (1955) confirmed the requirement of 1.5% of glycine in a diet containing approximately 20% of protein and 1.2% of serine. This quantity was not replaceable by diammonium citrate but was almost fully replaceable by DL-serine. The increase in growth on the addition of glycine to the diet was not due to the extra nitrogen in the glycine. A nutritional relation between glycine and serine, as previously reported in rats, was indicated for the chick. The arginine present in the optimal diet was approximately 6 grams per 16 grams of nitrogen.

A recent report on the isoleucine requirement of the laying hen indicates this to be 3.3 grams per 16 grams of nitrogen (E. C. Miller, 1954). The leucine requirement appears to be not more than 6.7 grams per 16 grams of nitrogen (Machlin, 1955). Further studies of the methionine requirement of the laying hen indicate that it is 0.3% of a diet containing 16.8% of protein or approximately 1.8 grams per 16 grams of nitrogen, in good agreement with previous estimates (Mehring, 1954).

Amino acids requirements of young swine have been listed in a National Research Council publication (Beeson, 1953a). It should be added that these are not correlated with the widely varying experimental protein levels with which they were determined, hence they do not apply uniformly to any one protein level. The threonine requirement of the weanling pig appears to be approximately 3 grams per 16 grams of nitrogen, in agreement with previous estimates (Beeson, 1953b). The valine requirement of the weanling pig is probably not more than 3.1 grams per 16 grams of nitrogen (Jackson, 1953). Limited data indicate that 2 grams of histidine per 16 grams of nitrogen is an adequate allowance for the young pig. It was demonstrated, by means of an amino acids diet, that pigs grew slowly or not at all when histidine was absent, but grew well when histidine was restored to the diet (Eggert, 1955). In studies on the amino acid requirement of the pig from weaning to about 100 lbs. weight, Pfander (1955) found that 5.0% lysine, 3.5% methionine plus cystine and 1.0% tryptophan in the dietary protein were adequate allowances.

Rose (1955d) has studied the replacement of methionine by cystine in the diet of man. Total sulfur-amino acid requirement for nitrogen balance of young adult males was supplied by 0.8 to 1.0 grams of methionine daily. This is somewhat lower than the previous estimate of 1.10 gram, which was, however, intended to provide the needs of individuals in the higher range of requirement. If an average requirement of 0.9 gram is assumed, then the figure for methionine plus cystine requirement of man, as listed in Table XVIII could be changed to 3.6, and would be in closer agreement with the value calculated from diets. Eighty to ninety per cent of the methionine requirements of adult man was found to be replaceable by cystine (Rose, 1955d) and 70 to 75% of the phenylalanine requirements by tyrosine (Rose, 1955e).

CHAPTER VIII

Protein and Amino Acid Consumption in the United States

Since the days of Magendie, Boussingault and Liebig the pendulum of enthusiasm about the proteins has swung from one extreme to another. Liebig's doctrine of the superior role of proteins has been challenged by Chittenden and others (*cf.* Mendel, 1923). The recommended dietary allowances (National Research Council, 1942) offer a compromise between these views, leaning, however, more towards Chittenden than Liebig. Nevertheless, the status of protein in human nutrition is still in the state so beautifully described by Lusk (1928). "Chittenden and Lusk crossed the English Channel together on March 20, 1918. Both had been taking for several weeks the greatly restricted British civilian diet of the time. The meat equivalent of four small chops a week had not been exceeded. On the naval transport 'military rations' were allowed and Lusk partook of a large slice of English cold roast beef to his delight and satisfaction. This sensation he attributed to the replenishment of the 'improvement quota' of his protein stores, while Chittenden was inclined to attribute the result to the appetite creating stimulus action of the sea air."

The U. S. Department of Agriculture has prepared an excellent survey of "Family Food Consumption in the United States" (1944). This survey gives the amounts and kinds of foods purchased by different economic groups in the United States during the spring of 1942. During this period the cost of food had increased from prewar levels but the annual income of a cetrain portion of the population was still very low; therefore, any nutritive deficiencies in the lowest income group may be greater than in the prewar years, although the economic and nutritional status of the average family had improved considerably.

The data in Tables XXIV to XXXI were calculated from information given in this U.S.D.A. publication using the analytical data presented in Table XXXIV. The analytical values in Table XXXIV are those which we believed to be the best available in 1950. Although there may be some changes in the amino acid figures, their order of magnitude is not expected to change appreciably.

The values given in Tables XXIV to XXXIII "are based on values of foods as they are purchased, losses in nutrients caused by preparation and household waste" when known must be subtracted. "The extent of such losses is not well known and probably is highly variable, consequently, it is impossible at present to estimate just how much the nutritive values of diets calculated from foods as they enter the Nation's kitchens overestimate the quantities actually consumed by families."

Amino Acids in Foods and Feeds

Practically all the nonessential or dispensable amino acids are found in animal tissue proteins and are required in the formation of body proteins. They may be regarded as physiologically indispensable. If the dispensable amino acids are not obtained in the food, they must be synthesized by the animal from other sources of carbon and nitrogen. The presence of the dispensable amino acids in the diet reduces the necessity of synthesizing them from the indispensable amino acids. Thus the dispensable amino acids exert a general saving or "sparing" action on the indispensable amino acids. This sparing action is of much importantce in practical nutrition.

Specific examples of this sparing action may be cited. Methionine will furnish part of the materials needed by the animal to synthesize cystine. Consequently, cystine is not necessary in the diet if there is sufficient excess of methionine to meet the requirements for both methionine and the synthesis of cystine. If cystine is present in the diet, however, the necessity for conversion of methionine to cystine is correspondingly diminished, and a lower level of methionine will suffice; more than one report of a high methionine requirement may be traceable to the use of an experimental diet which was low in cystine. The sparing action of cystine is of practical importance, especially for young animals, because it is not easy to provide more than the minimum requirement for methionine, itself, by the usual selection of feedstuffs. A similar relation exists between phenylalanine and tyrosine. Tyrosine is synthesized from phenylalanine, but is indispensable only when there is enough phenylalanine to meet the needs for both amino acids. It appears that arginine, glutamic acid and proline are interconverted in the rat (Womack, 1947).

There is ample evidence that forced synthesis of dispensable amino acids from indispensable amino acids will not proceed without considerable waste. Thus amino acids requirements studies based on diets which contain only the indispensable ones are certain to give erroneously high results.

TABLE XXIV

Average Quantity of Each Essential Amino Acid Contributed by Specified Food Groups to the *Poorest Urban Class* (Annual Income 0-499 Dollars) During the Spring of 1942*
(Calculated in grams per capita per day)

	All Foods	*Milk, Cheese, Ice Cream, etc.*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	g.	g.	g.	g.	g.	g.	g.	g.
Total protein	74.00	14.30	15.00	23.20	6.00	6.00	7.50	2.30
Arginine	4.23	0.61	1.12	0.84	0.38	0.56	0.52	0.20
Histidine	1.69	0.39	0.43	0.46	0.13	0.10	0.16	0.02
Lysine	4.00	1.19	1.21	0.37	0.43	0.24	0.43	0.13
Tyrosine	3.45	0.83	0.51	1.11	0.27	0.26	0.40	0.02
Tryptophan	0.91	0.22	0.20	0.21	0.09	0.05	0.14	0.00
Phenylalanine	4.05	0.85	0.78	1.34	0.38	0.31	0.34	0.05
Methionine + Cystine	2.98	0.61	0.69	0.79	0.39	0.16	0.32	0.02
Threonine	2.81	0.60	0.69	0.74	0.25	0.16	0.31	0.05
Leucine	6.47	1.50	1.16	2.31	0.55	0.38	0.50	0.07
Isoleucine	4.20	1.16	0.94	1.06	0.48	0.25	0.27	0.04
Valine	4.17	1.17	0.87	1.06	0.44	0.24	0.33	0.06

* From "Family Food Consumption in the United States," U.S.D.A. Publication No. 550, 1944.

TABLE XXV

Percentage of Each Essential Amino Acid Contributed by Food Groups Calculated From Table XXIV
($0-$499 per annum income)
Urban

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	%	%	%	%	%	%	%	%
Total protein	100	19	20	31	8	8	10	3
Arginine	100	14	27	20	9	13	12	5
Histidine	100	23	25	27	8	6	10	1
Lysine	100	30	30	9	11	6	11	3
Tyrosine	100	24	15	32	8	8	12	2
Tryptophan	100	24	22	23	10	6	15	0
Phenylalanine	100	21	19	33	9	8	8	1
Methionine + Cystine	100	21	23	27	13	5	11	1
Threonine	100	21	25	26	9	6	11	2
Leucine	100	23	18	36	9	6	8	1
Isoleucine	100	28	22	25	11	6	6	1
Valine	100	28	21	25	11	6	8	1

TABLE XXVI

AVERAGE QUANTITY OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY SPECIFIED FOOD GROUPS TO THE *Urban Middle Class* (ANNUAL INCOME $2,500 TO $2,999) DURING THE SPRING OF 1942*
(Calculated in grams per capita per day)

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	%	%	%	%	%	%	%	%
Total protein	94.00	20.00	31.30	21.00	7.60	3.80	9.50	1.00
Arginine	5.63	0.85	2.41	0.77	0.49	0.36	0.66	0.09
Histidine	2.31	0.55	0.91	0.42	0.16	0.06	0.20	0.01
Lysine	5.82	1.65	2.53	0.34	0.55	0.15	0.54	0.06
Tyrosine	4.26	1.17	1.06	1.00	0.34	0.17	0.51	0.01
Tryptophan	1.23	0.31	0.41	0.19	0.11	0.03	0.18	
Phenylalanine	5.07	1.20	1.53	1.22	0.48	0.19	0.43	0.02
Methionine + Cystine	4.03	0.85	1.44	0.73	0.49	0.10	0.41	0.01
Threonine	3.89	0.84	1.44	0.67	0.33	0.10	0.39	0.02
Leucine	8.19	2.10	2.41	2.10	0.70	0.24	0.61	0.03
Isoleucine	5.69	1.62	0.97	0.97	0.61	0.15	0.34	0.02
Valine	5.57	1.64	1.82	0.96	0.55	0.15	0.42	0.03

* From "Family Food Consumption in the United States," U.S.D.A. Publication No. 550, 1944.

TABLE XXVII

PERCENTAGE OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY FOOD GROUPS CALCULATED FROM TABLE XXVI
($2,500-$2,999 per annum income)
Urban

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	%	%	%	%	%	%	%	%
Total protein	100	21	33	22	8	4	10	1
Arginine	100	15	43	14	9	6	12	2
Histidine	100	24	39	18	7	3	9	0
Lysine	100	28	44	6	10	3	9	1
Tyrosine	100	28	25	24	8	4	12	0
Tryptophan	100	25	33	15	9	2	15	0
Phenylalanine	100	24	30	24	10	4	9	0
Methionine + Cystine	100	21	36	18	12	2	10	0
Threonine	100	22	37	17	9	3	10	1
Leucine	100	26	29	26	9	3	7	1
Isoleucine	100	28	35	17	11	3	6	0
Valine	100	29	33	17	10	3	8	1

TABLE XXVIII

AVERAGE QUANTITY OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY SPECIFIED FOOD GROUPS TO THE *Poorest Farm Class* (ANNUAL INCOME 0-499 DOLLARS) DURING THE SPRING OF 1942*

(Calculated in grams per capita per day)

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	g.	g.	g.	g.	g.	g.	g.	g.
Total protein	90.00	25.20	12.60	32.40	5.40	6.30	6.50	1.80
Arginine	4.78	1.07	0.97	1.18	0.35	0.59	0.46	0.16
Histidine	2.08	0.69	0.37	0.65	0.11	0.11	0.14	0.01
Lysine	4.74	2.09	1.02	0.52	0.39	0.25	0.37	0.10
Tyrosine	4.31	1.47	0.43	1.54	0.24	0.27	0.35	0.01
Tryptophan	1.11	0.40	0.16	0.30	0.08	0.05	0.12	0.00
Phenylalanine	5.00	1.51	0.62	1.88	0.34	0.32	0.27	0.04
Methionine + Cystine	3.62	1.08	0.58	1.13	0.35	0.18	0.28	0.02
Threonine	3.37	1.06	0.58	1.02	0.23	0.17	0.27	0.04
Leucine	8.25	2.65	0.97	3.24	0.50	0.40	0.43	0.06
Isoleucine	5.27	2.04	0.79	1.49	0.43	0.26	0.23	0.03
Valine	5.27	2.08	0.73	1.48	0.39	0.25	0.29	0.05

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* From "Family Food Consumption in the United States," U.S.D.A. Publication No. 550, 1944.

TABLE XXIX

PERCENTAGE OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY FOOD GROUPS CALCULATED FROM TABLE XXVIII

($0-$499 per annum income)

Farm

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	%	%	%	%	%	%	%	%
Total protein	100	28	14	36	6	7	7	2
Arginine	100	22	20	25	7	12	10	3
Histidine	100	33	18	31	5	5	7	1
Lysine	100	44	22	11	8	5	8	2
Tyrosine	100	34	10	36	6	6	8	0
Tryptophan	100	36	14	27	7	5	11	0
Phenylalanine	100	30	12	38	7	6	6	1
Methionine + Cystine	100	30	16	31	10	5	8	1
Threonine	100	32	17	30	7	5	8	1
Leucine	100	32	12	39	6	5	5	1
Isoleucine	100	39	15	28	8	5	4	1
Valine	100	39	14	28	8	5	6	1

TABLE XXX

AVERAGE QUANTITY OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY SPECIFIED FOOD GROUPS TO THE *Farm Middle Class* (ANNUAL INCOME $1,000-$1,499) DURING THE SPRING OF 1942[*]
(Calculated in grams per capita per day)

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	g.	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*
Total protein	95.00	25.60	19.00	25.70	7.60	6.60	8.60	1.90
Arginine	5.38	1.10	1.46	0.94	0.49	0.62	0.60	0.17
Histidine	2.24	0.71	0.55	0.52	0.16	0.11	0.18	0.01
Lysine	5.49	2.12	1.54	0.41	0.55	0.27	0.49	0.11
Tyrosine	4.48	1.50	0.65	1.23	0.34	0.29	0.46	0.01
Tryptophan	1.21	0.40	0.25	0.23	0.11	0.06	0.16	0.00
Phenylalanine	5.18	1.52	0.93	1.47	0.48	0.35	0.39	0.04
Methionine + Cystine	3.93	1.09	0.87	0.89	0.50	0.19	0.37	0.02
Threonine	3.66	1.08	0.87	0.81	0.33	0.18	0.35	0.04
Leucine	8.38	2.69	1.46	2.57	0.70	0.43	0.57	0.06
Isoleucine	5.68	2.07	1.20	1.18	0.61	0.28	0.31	0.03
Valine	5.64	2.11	1.10	1.18	0.56	0.26	0.38	0.05

[*] From "Family Food Consumption in the United States," U.S.D.A. Publication No. 550, 1944.

TABLE XXXI

PERCENTAGE OF EACH ESSENTIAL AMINO ACID CONTRIBUTED BY FOOD GROUPS FROM TABLE XXX
($1,000-$1,499 per annum income)
Farm

	All Foods	*Milk, Cheese, Ice Cream*	*Meat, Poultry, Fish*	*Grain Products*	*Eggs*	*Beans, Nuts*	*Potatoes, Vegetables, Fruits*	*Fats, Oils, etc.*
	%	%	%	%	%	%	%	%
Total protein	100	27	20	27	8	7	9	2
Arginine	100	20	27	17	9	12	11	3
Histidine	100	32	25	23	7	5	8	0
Lysine	100	39	28	7	10	5	9	2
Tyrosine	100	33	15	28	8	6	10	0
Tryptophan	100	33	21	19	9	5	13	0
Phenylalanine	100	29	18	28	9	7	8	1
Methionine + Cystine	100	28	22	23	13	5	9	0
Threonine	100	30	24	22	9	5	10	0
Leucine	100	32	17	30	8	5	7	1
Isoleucine	100	36	21	21	11	5	6	0
Valine	100	37	20	21	10	5	7	1

For the quantitative provision of amino acid requirements it is necessary to have information on the amino acid content of foods to be used. Such data have accumulated rapidly in recent years and it is possible to make up tables of composition giving the expected or average amino acid content of practical feedstuffs. These tables are, of course, subject to the errors due to usual variations in natural products and to deviation in analytical procedure. Similar limitations have also plagued nutrition workers in the cases of the vitamins. The uncertainties of such tables must always be kept in mind; yet it is surprising how well the tables usually work out.

It is fortunate that with a few exceptional instances, practical foods which provide adequate levels of five certain amino acids will meet the requirements of the animal for all others. The tables of amino acids in feedstuffs which are found in Part B may, therefore, be simplified for practical purposes to arginine, lysine, methionine, cystine and tryptophan. These could be considered a "critical" list from a practical standpoint. Although not "indispensable," cystine is included in this list because the full requirements of young animals for total sulfur amino acids (methionine plus cystine) cannot usually be met by methionine alone in practical feedstuffs. However, to translate the values in the tables into amino acid content of actual foods and feeds it is only necessary to multiply by the fraction of the food which is in protein form.

For the purposes of this discussion only four of the diets of the many economic groups given in the U.S.D.A. report are analyzed. These are:

Group 1—Urban Families, annual income less than $500; Tables XXIV and XXV.
Group 2—Urban Families, annual income from $2500 to $2999; Tables XXVI and XXVII.
Group 3—Farm Families, annual income less than $500; Tables XXVIII and XXIX.
Group 4—Farm Families, annual income from $1000 to $1499; Tables XXX and XXXI.

Tables XXIV to XXXI show the absolute and percentage contributions made by each of the major foodstuffs to the daily per capita intake of protein, arginine, histidine, lysine, tyrosine, tryptophan, phenylalanine, cystine plus methionine, threonine, leucine, isoleucine and valine. These results are summarized in Table XXXII, where the values from our earlier calculations are also given (Block and Boll-

TABLE XXXII

AVERAGE DAILY PER CAPITA CONSUMPTION OF PROTEINS AND OF ESSENTIAL AMINO ACIDS IN THE UNITED STATES

	Urban Groups 1942		*Farm Groups 1942*		*Total Population 1937-1941 Average*
	1 *Income $0-$499*	*2* *Income $2,500-$2,999*	*3* *Income $0-$499*	*4* *Income $1,000-$1,499*	
	g.	*g.*	*g.*	*g.*	*g.*
Total protein	74.0	94.0	90.0	95.0	89.0
Arginine	4.2	5.6	4.8	5.4	4.7
Histidine	1.7	2.3	2.1	2.2	2.0
Lysine	4.0	5.8	4.7	5.5	5.2
Tyrosine	3.5	4.3	4.3	4.5	3.9
Tryptophan	0.9	1.2	1.1	1.2	1.1
Phenylalanine	4.1	5.1	5.0	5.2	4.7
Methionine + Cystine	3.0	4.0	3.6	3.9	4.1
Threonine	2.8	3.9	3.4	3.7	3.6
Leucine	6.5	8.2	8.3	8.5	(12.6)*
Isoleucine	4.2	5.7	5.3	5.7	(3.7)*
Valine	4.2	5.6	5.3	5.6	(3.9)*

* The methods used for leucine, isoleucine, and valine in 1942-43 gave high values for leucine and low values for isoleucine and valine. They were useful, however, for comparative purposes but have now been replaced by superior procedures.

ing, 1945). It will be seen that in spite of certain improvements in the American diet and changes in the methods for the determination

TABLE XXXIII

PER CENT DEVIATION OF EACH ESSENTIAL AMINO ACID FROM THE PATTERN OF THE URBAN $2,500-$2,999 INCOME GROUP 2

	Urban Group 1 $0-$499	*Farm Group 3 $0-$499*	*Farm Group 4 $1,000-$1,499*
	%	%	%
Arginine	− 3.7	−10.7	−5.2
Histidine	− 5.6	− 5.6	−3.7
Lysine	−10.1	−14.2	−6.4
Tyrosine	+ 2.2	+ 5.6	+4.4
Tryptophan	− 4.8	− 5.4	−2.3
Phenylalanine	+ 1.2	+ 3.0	+1.5
Methionine + Cystine	− 4.9	− 5.8	−3.2
Threonine	− 6.6	− 9.0	+6.6
Leucine	+ 0.2	+ 5.1	+2.8
Isoleucine	− 5.0	− 3.0	−0.9
Valine	− 3.9	− 1.0	+0.5

TABLE XXXIV

Essential Amino Acids Yielded by the Hydrolysis of 100 g. of Protein (N × 6.25) of Various Foods Calculated to 16.0 g. of Nitrogen Per 100 g. of "Crude Protein"

	Milk	*Cheese*	*Meat, Poultry, Fish*	*Wheat Gluten*	*Corn Grits*	*Eggs*	*Beans° Nuts°*	*Potatoes, Vegetables, Fruits*	*Fats, Oils*
	g.	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*	*g.*
Arginine	4.3	4.2	7.7	3.9	3.1	6.4	9.4	7.0	8.7
Histidine	2.6	3.0	2.9	2.2	1.6	2.1	1.7	2.1	0.9
Lysine	8.2	8.5	8.1	2.0	0.8	7.2	4.0	5.7	5.8
Tyrosine	5.3	6.9	3.4	3.8	6.7	4.5	4.4	5.4	0.7
Tryptophan	1.6	1.5	1.3	1.0	0.7	1.5	0.8	1.9	0.0
Phenylalanine	5.7	6.5	4.9	5.5	6.4	6.3	5.1	4.5	2.1
Cystine	1.0	0.4	1.3	1.9	1.1	2.4	1.4	2.0	0.1
Methionine	3.4	3.6	3.3	1.5	2.5	4.1	1.4	2.3	0.8
Threonine	4.5	3.6	4.6	2.7	4.1	4.3	2.7	4.1	2.1
Leucine	11.3	8.9	7.7	7.5	15.0	9.2	6.4	6.6	3.1
Isoleucine	8.5	7.3	6.3	3.7	6.4	8.0	4.1	3.6	1.7
Valine	8.5	7.7	5.8	4.2	5.3	7.3	4.0	4.4	2.8

° These values are calculated from equal portions of protein derived from peas and peanuts. If soybeans were included, the lysine content would be increased.

of the essential amino acids, the over-all pattern of the daily per capita intake of amino acids has not changed appreciably except in the case of leucine, isoleucine and valine. This difference is due to the superiority of the biological methods over the older chemical procedure for estimation of these three amino acids.

Since Mendel (1923) pointed out that protein nutrition is in essence the nutrition of essential amino acids, it has been recognized that the quality as well as the quantity of dietary protein is important. Table XXXIII shows the per cent deviation of each essential amino acid in one urban and two rural groups, when urban group No. 2 (annual income $2500 to $2999) was set as the arbitrary standard. It should be noted that the values given in Table XXXII are all calculated on the basis of 94 g. of protein. This was done in order to ascertain the quantitative differences in the diets of the groups under investigation.

TABLE XXXV

Amino Acid Intakes During Pregnancy

	Conventional Diet	*Ovo-lacto-Vegetarian Diet*
	g./day	*g./day*
Nitrogen	10.4	8.3
Arginine	3.3	2.9
Histidine	1.5	1.6
Lysine	4.1	3.6
Tryptophan	0.7	0.7
Phenylalanine	2.8	3.1
Methionine	1.8	1.5
Threonine	2.9	2.6
Leucine	5.4	5.3
Isoleucine	3.5	3.3
Valine	3.8	3.8

Sheft (1952) analyzed the diets of pregnant women eating either a conventional diet containing meat or those restricted to eggs, milk, cereals and fruits. These results are given in Table XXXV.

Mitchell and Hamilton (1929) say "The prevailing dietary habits in this country, for no obvious reason at all, are considered merely adequate; hence, the possibility of increased human efficiency and happiness following super-adequate nutrition." Whether the protein and amino acid intake of any or all of the four groups discussed in this report is deficient, adequate or super-adequate, must be left for future investigation; however, the calculations show that the "average" American eating about 90 g. of protein per day, receives approximately 5 g. of arginine, 2 g. of histidine, 5 g. of lysine, 4 g. of tyrosine, 1 g. of tryptophan, 5 g. of phenylalanine, 4 g. of methionine plus cystine, almost 4 g. of threonine, 8 g. of leucine, 5 g. of isoleucine and 5 g. of valine (*cf.* Chapter VII, especially Table XII).

The data also indicate if there are protein deficiencies especially among the lowest income groups, judicious enrichment of appropriate foods with certain amino acids, especially lysine and threonine, or with sources abundantly supplied with these and other amino acids, should add much to successful nutrition without appreciably increasing the cost of the diet.

The data in this book should also permit physicians and dietitians who wish to increase protein (*i.e.*, amino acid) intake to choose those proteins and foods which contain an excess of essential amino acids which may be presumed from a knowledge of the usual food intake, to be inadequatelv supplied in the diet of their patients.

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Part B

THE AMINO ACID COMPOSITION OF PROTEINS

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PART B

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	Source	*Method*	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*
WHOLE BLOOD										
Blood Flour					4.3	6.1	10.1	2.5	1.3	7.1
Blood Flour, an average					4.2	5.6	7.4	2.2	1.3	7.3
Blood Flour, a range: Chicken, Duck, Turkey	Fowl	M			4.7–	5.4–	8.3–			5.8–
Blood Flour					5.1	5.9	10.0			6.7
Blood Flour, a range: Cat, Dog, Rabbit, Rat, Mouse	Mammal	M			3.6–	5.7–	9.2–			6.6–
					4.4	7.5	11.0			8.0
Blood Flour	Swine	M			4.0	6.3	9.0			6.5
Blood Flour	Swine	M			5.2	9.2	9.2	2.5	1.5	7.3
Blood Flour	Swine	M	11.8		4.8	6.1	8.9	2.9	1.3	6.8
FIBRIN										
Fibrin		M								
Fibrin	Bovine	C	16.3			2.0				
Fibrin, an average	Bovine	CM	16.77		7.2	2.5	8.7	5.6	3.5	4.5
Fibrin, an average	Horse	CM			6.7	2.5	7.6		1.2	3.5
Fibrin	Human	C	16.89			2.2				
Fibrin	Sheep	C	(17.5)			2.0		3.0		
Fibrin	Swine	C	(17.5)			2.1		3.1		
Fibrinopeptide		PX	M. R.		1		1			1
SERUM PROTEINS										
Total—Normal										
Total—Normal	Bovine	C	14.6		5.7	1.9	7.9	5.0	1.8	5.0
Total—Normal	Dog	C	13.1	1.19	5.7	1.8	8.5	5.7	1.8	
Total—Normal	Human	C	(16.0)		5.9	3.2	9.1	5.1	1.4	3.4
Tot.—Normal, an avge.	Human ♀	C	14.7		2.3	2.8	3.7	11.3	1.7	2.0
Tot.—Normal, an avge.	Human	C			5.5	2.7	7.5	4.9	1.4	5.4
Total—Normal	Human	CPX	(16.0)		5.3	2.8	10.5	5.1	0.9	3.7
Total—Normal	Human	M	(16.0)		4.5	2.5	9.0			10.5
Tot.—Normal, on avge. of 28 individuals	Human	M	16.0		4 6	2.6	9.2			8.8
Total—Normal	Rat	M			4.9	2.1	7.0			4.7
Total—Normal, Various animals		C	14.5	1.32	5.7	2.4	7.7	5.4	1.7	5.5
Total—Pathological										
Cachetic Dystrophy (Avg. of 16 cases)	Human	CPX	(16.0)		3.8	2.6	5.8	7.4	1.9	3.9
Edematous Dystrophy (Before nourishment)	Human	C				2.9	6.0	7.1		3.6
Edematous Dystrophy (After nourishment)	Human	C				3.0	8.5	5.1		
Lipophilic Dystrophy (Avg. of 5 cases)	Human	C	(16.0)		2.9	3.2	5.7	1.7	1.4	2.5
Melanosarcoma	Human	CPX	(16.0)		1.7	2.4	19.9	9.7	5.0	8.2
Nephrosis	Human	M	(16.0)		3.7		6.1			
β-Plasmacytoma	Human	M	(16.0)		4.4		5.5			
γ-Plasmacytoma	Human	M	(16.0)		5.2		8.5			
SERUM ALBUMINS										
Normal										
Normal	Bovine	C	16.22			3.3				
Normal	Bovine	C	16.1							
Normal	Bovine	CM	15.79		6.3	3.9	12.6	5.5	0.6	6.3
Normal	Bovine	M	16.1		5.7	3.7	11.4	4.9		6.2
Normal	Bovine	M	16.1		6.0	4.1	11.6	5.3	0.6	6.3
Normal	Bovine	M	16.1		5.8	3.7	11.5	4.9		6.3
Normal	Bovine	M	(16.0)		5.6	3.8	11.9	4.4	0.6	6.3
Normal, mean value	Bovine	PX	(16.0)		6.0	4.1	12.8	5.2	0.6	6.4
Normal, an average val.	Bovine	C			5.5	2.9	8.4	4.9	1.1	
Normal, an average val.	Dog	C			6.1	3.1	10.4	5.9	1.2	7.5
Normal, an average val.	Horse	CM		1.62	4.6	2.6	8.3	5.2	1.1	5.6
Normal	Horse	PX	(16.0)		7.7	3.1		4.2		
Normal	Human	C	(16.0)							
Normal	Human	C	16.45			3.1				
Normal	Human	CM	15.19		6.5	3.7	13.0	4.9	0.2	8.2
Normal	Human	CPX	(16.0)		6.1	3.5	11.5	3.5	0.5	2.0
Normal	Human	M	(16.0)		5.9	3.2	12.0			7.6
Normal	Human	PX			5.9	3.7	12.4	5.2		
Normal	Human	PX	(16.0)		6.3		12.0	4.9		5.6
Nor., range of 20 samples	Human	CPX			5.2–	2.8–	11.5–	4.1–	0.2–	7.4–
					6.3	3.7	13.7	4.8	0.5	8.6
Normal, an average val.	Human	C			5.3	2.9	11.8	4.7	0.7	7.9
Normal	Rabbit	M			5.3	3.0	8.0	6	0.4	11.9
Normal	Rabbit	MPX	(16.0)				9.8			
Pathological										
Hodgkins Disease	Human	M	(16.0)		5.9	3.3	12.5			7.4
Leukemia	Human	M	(16.0)		5.9		12.5			7.9
Liver Cirrhosis	Human	M	(16.0)		6.0	3.5	12.0			7.6
Nephrosis, range 4 cases	Human	M	(16.0)		6.0–	3.0–	12.0–			7.6
					6.3	3.1	12.5			
Nephrosis, urine albumin, avg. of 2 cases	Human	M	(16.0)		6.1	3.5	12.3			7.5
Plasmacytoma	Human	CPX	(16.0)		1.2	3.1	9.4	5.2	1.0	3.6

D URINE PROTEINS

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

T	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
3		4.7	12.0	1.5	9.0							William, H.H., '55
1		4.1	11.9	1.2	7.8							*Cf* B. & B, '51 *
2–		4.6–	10-11	4.4–	7.0–	9.5–	9.8–	2.8–				
6		5.3		4.9	8.0	10.0	11.0	4.4				Dunn, '49c
3–		4.5–	11-13	1.7–	7.2–	7.6–	11-14	3.5–				Dunn, '49c
8		6.3		3.6	8.8	12.0		6.9				
8		3.4	12.3	1.2	8.1	9.0	12.5	5.1				Camien, '49
2	8.4	2.3	13.7	1.2	10.7	10.7	16.1	5.5	1.0	4.9		Agren, '51
		4.4	9.6	1.1	8.3							Brinegar, '50b
								5.3				McCoy, '53a
												Vickery, '44
6	12.8	7.3	10.7	5.8	4.1	13.6	14.8	5.3	4.0	5.3		*Cf*. B & B, '51
0			7.0	2.8	6.0	15.4						*Cf*. B & B, '51
												Vickery, '44
												Cf. B & B, '51
												Cf. B & B, '51
	2	1		1	1	3	4	4	1			Lorand, '52
7		6.3	10.1	3.4	7.5	14.2						*Cf*. B & B, '51
												Cf. B & B, '51
3			24.2									Müting, '49a & Gülzow, '49
6	1.4	2.1	25.0	2.0	3.2							*Cf*. B & B, '51
9		6.3	18.0	2.7	6.0							*Cf*. B & B, '51
1	5.4	6.9	10.9	1.8	5.7	14.7	9.1	2.9	5.3	5.0	0	Müting, '51/'52
		4.8	9.6	3.6	5.9							Kazmeier, '53
		4.8	9.8	3.6	5.9							Esser, '55
3		4.5	7.1	3.9	6.9							Solomon, '51
0		6.3	10.1	3.4	7.5	14.2						*Cf*. B & B, '51
8			15.5									Gülzow, '49 ; '51/'52
9			16.3									Gülzow, '49
9			19.6									Gülzow, '49
6			16.2									Gülzow, '49 ; '51/'52
4	4.1	5.0	12.0	1.5	4.5	14.4	8.0	3.0	5.0	4.5		Müting, '51/'52
		4.7	7.0	5.2								Kazmeier, '53
		6.7	8.8	3.9								Kazmeier, '53
		6.4	8.5	4.7								Kazmeier, '53
												Vickery, '44
										5.0	0.1	Troll, '53b
.8	4.6	6.6	13.9	2.9	6.6	17.0	10.8	1.9		5.7		Brand, '47
.8	4.0	5.7	11.9	2.6	6.1	16.1	9.1	1.7	5.0	4.6		Lewis, '50
.8	4.9	6.7	12.2	3.0	6.0	17.5	10.5	2.0		5.5		*Cf*. B & B, '51
.8		7.6	12.0	3.7	8.3	16.2	10.2	1.7		4.7		Mohammed, '49
.9	3.8	5.5	11.4	2.9	6.0	22.4	10.1	1.8		4.7		McClure, '53b
.8	4.4	5.9	11.9	2.6	5.5	17.4	10.6	1.9	6.8	4.4		Redfield, '52
.5						17.1	10.0	2.0			0.0	*Cf*. B & B, '51
.5	9.5	7.7	10.1	2.4	8.5							*Cf*. B & B, '51
												Cf. B & B, '51
.0	4.2	4.0	16.5		4.8	12.4	9.5	2.2	6.3	4.5		De Wael, '54
												Emmrich, '50
												Vickery, '44
.3	3.9	5.3	12.5	1.8	8.1	18.3	11.0	1.7		5.4		Brand, '47
.7	4.0	5.2	11.5	1.7	6.0	17.2	10.1	2.1	5.5	4.3	0.2	Müting, '52b
.3		5.8	12.9	1.9	8.0							Schreier, '51
.1	4.5	5.9			5.6	16.5	10.2	1.9	6.0	5.5		Wellington, '52
	2.6	3.1	12.1		6.3	17.1	9.9	1.0	8.1	4.4		Pernis, '53
.8	3.9–	5.2–	9.8–	1.2–	6.9–	12.3–	8.0–	1.2–	6.5–	5.2–		Müting, '54
.3	4.6	6.1	11.1	1.8	8.7	15.7	9.5	1.7	7.9	6.3		
						17.0	10.3	1.6				*Cf*. B & B, '51
.3		4.1	9.5	3.2	6.6	9.9	7.5	2.3	4.1	4.1		Schneiderman, '53
					7.8			3.1				Campbell, '52
.3		5.9	12.7	2.0	7.7							Schreier, '51
.4		5.7	12.6	2.1	7.9							Schreier, '51
.3		5.8	12.5	1.7	7.7							Schreier, '51
.3		5.7–	12.6–	1.8–	7.5–							Schreier, '51
.5		5.8	13.1	2.1	8.0							
.3		5.8	13.0	1.9	8.0							Schreier, '51
.5	6.5	5.0	10.4	2.2	5.0	21.8	11.1	2.7	5.0	5.6	0.7	Müting, '52b

	Source	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	PHE
SERUM GLOBULINS										
Normal										
Normal	Bovine	C	14.2		5.3	2.5	6.3	5.7	1.9	
Normal	Dog	C			6.6	3.1	6.0	5.6	3.0	6.7
Normal, an average	Horse		15.8	1.09	5.1	1.4	5.2	5.4	2.0	
Normal	Human	CPX	(16.0)		4.5	2.2	8.5	6.5	2.2	4.1
Normal	Human	C	(16.0)							
Normal, an average	Human	C	15.5	0.63	4.1	1.9	9.1	5.9	2.1	6.9
Normal	Rabbit	MPX	(16.0)				6.6			
γ-Cryoglobulin		X	(16.0)		1.1	3.2	12.0	6.8	0.9	6.2
α-Globulin, range of 20 samples	Human	CPX			4.2–5.2	2.7–3.4	8.1–9.5	3.6–4.6		4.3–5.3
β-Globulin		PX	(16.0)		5.7		6.1	6.2		6.0
β-Globulin, range of 20 samples	Human	CPX			5.0–6.0	2.2–3.1	6.9–7.8	4.8–5.9		4.5–5.3
γ-Globulin	Human	C	14.2							
γ-Globulin	Human	CM	15.41		5.0	2.6	8.4		3.0	4.8
γ-Globulin		PX	(16.0)		5.5		5.6	5.4		4.8
γ-Globulin		X	(16.0)		5.5	2.4	7.2	5.5	3.6	5.1
γ-Globulin, range of 20 samples		CPX			2.3–2.8	2.2–2.7	7.9–9.0	5.8–6.8	2.2–2.7	4.9–6.0
Pathological										
Antibodies to *Pneumococcus* I, VII, VIII, XIV		X			5.0	1.5	6.5	6.6	2.7	5.5
Epithelioma, Cancerous—Protein "A"	Rat	PX			5.5	4.4	14.4	4.1	1.2	4.5
Epithelioma, Cancerous—Protein "C"	Rat	PX			5.6	6.8	8.1	2.7	1.0	6.6
Globulin-β, from Macroglobulinemia		PX	(16.0)		4.9–5.2		3.8–4.1	4.0–6.4		5.2
Globulin-γ, from Macroglobulinemia		PX	(16.0)		4.6–5.3		3.4–3.5	4.5–5.1		4.5–5.5
Multiple Myeloma, range of 4 cases		X	(16.0)		2.7–5.0	2.0–2.2	5.3–7.4	4.4–6.7	1.5–2.8	4.4–4.9
Macroglobulin, isol. from plasma	Human	M	16.2		5.5	4.1	6.0	5.0	1.2	2.7
Macroglobulin β_1		PX	(16.0)		4.9		3.8	4.0		5.2
Macroglobulin β_1		PX	(16.0)		5.2		5.1	6.4		5.2
Macroglobulin-γ		PX	(16.0)		5.3		3.5	4.5		5.5
Macroglobulin-γ		PX	(16.0)		4.6		3.4	4.1		4.5
Plasmacytoma	Human	CPX	(16.0)		2.2	2.6	7.9	8.2	4.1	6.6
GLOBINS AND HEMOGLOBINS										
Normal										
Globin, an average	Bovine	CM	16.0	0.50	3.4	7.8	8.1	2.9	2.0	7.8
Globin	Human	C	14.51							
Globin	Human	CPX	(16.0)		2.0	6.1	7.5	2.2	1.5	4.5
Globin	Human	CPX	(16.0)		3.0	7.6	7.0	2.7	1.3	5.4
Hemoglobin	Bovine	CM	(16.7)		3.3	7.1	8.3	2.0	1.0	7.0
Hemoglobin, fetal	Bovine	CM	(16.7)			6.2				
Hemoglobin	Cat					8.3				
Hemoglobin	Dog		(16.7)		4.0	7.4	8.5			8.0
Hemoglobin, an average	Horse	CM	(16.7)	0.43	3.5	7.6	8.3	2.9	2.2	7.1
Hemoglobin, fetal	Human	PX			3.8	8.3	11.3	3.1	1.6	8.0
Hemoglobin, fetal	Human	X			3.1	7.1	10.2	3.3	1.5	7.5
Hemoglobin, fetal	Human	X	(16.7)		3.2	7.1	9.4	3.0		7.5
Hemoglobin, fetal	Human				3.0	7.3	10.8	2.9		7.9
Hemoglobin, an average	Human	C			3.9	7.6				8.5
Hemoglobin	Human	PX			4.0	8.9	11.4	3.5	1.3	8.1
Hemoglobin	Human	X			3.3	8.0	10.2	4.1	1.1	7.5
Hemoglobin	Human	X	(16.7)		3.2	7.9	9.5	3.7		7.5
Hemoglobin	Human	X	(16.7)		3.3	8.1	10.2	2.8	1.9	9.2
Hemoglobin	Human	PX			3.5	8.5	10.6	2.9	2.0	9.6
Hemoglobin	Human				2.7	8.2	8.7	3.5		8.5
Hemoglobin, an average	Sheep	C			3.8	7.8	7.9	2.1		7.6
Hemoglobin, an average	Swine	C						2.0	1.1	7.1
Hemoglobin, an average	Turtle	CM	15.5		2.8	4.6		2.6	1.5	7.0
Pathological										
Hemoglobin, Homozygous Hemoglobin-C in disease	Human	X	(16.7)		3.3	8.2	10.4	3.7		7.7
Hemoglobin, Melanosarcoma	Human	CPX	(16.0)		1.5	9.1	6.2	11.7	5.0	12.9
Hemoglobin, Plasmacytoma	Human	CPX	(16.0)		1.3	7.9	7.4	7.2	3.1	4.1
Hemoglobin, Sickle Cell Anemia	Human	X	(16.7)		3.2	8.0	9.3	3.7		7.8
Hemoglobin, Sickle Cell Anemia	Human	X			3.2	8.0	9.1	2.8	1.5	7.2

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

SER	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
											Cf. B & B, '51
	6.3										*Cf.* B & B, '51
							5.0	2.0			*Cf.* B & B, '51
6.3	8.5	10.5	2.5	8.1	12.1	8.0	4.0	5.1	4.8		Müting, '52b
											Emmrich, '50
	5.0	17.6	0.2	10.5							*Cf.* B & B, '51
				9.1			2.8				Campbell, '51
11.8	9.3	10.4	2.8	7.5	13.7	9.7	4.4	3.7	9.1		Dustin, '54b
4.9–5.7	4.7–5.6	11.7–13.9	1.5–2.1	5.2–6.0	19.9–24.1	8.0–0.1	2.9–3.7	6.8–7.8	4.5–5.7		Müting, '54
7.0	4.4	12.1		7.8	13.4	8.8	3.4	5.2	7.8		Wunderly, '54b & Pernis, '54b
6.3–7.0	5.1–5.8	7.8–9.0	3.4–4.2	6.3–7.1	13.8–16.8	7.8–8.5	4.2–5.5	7.2–8.3	6.2–7.3		Müting, '54
		8.2	3.3	10.1							*Cf.* B & B, '51
11.8	8.7	9.6	2.8	10.1	12.2	9.1	4.4		8.4		Brand, '47
9.7	6.0	9.3		7.8	12.2	9.5	3.3	4.3	8.4		Pernis, '54b & Wunderly, '54b
10.0	7.2	8.3	2.4	7.3	11.8	8.5	4.2	4.1	6.2		Dustin, '54b
9.0–11.6	6.9–8.2	9.2–10.5	1.9–2.8	8.0–9.3	9.9–12.2	6.9–8.2	3.2–3.9	7.5–8.5	7.8–9.0		Müting, '54
10.9	13.2	7.9	4.4	10.2	11.8	9.7	5.7	5.7	8.6		Smith, E. L., '55b
4.0	3.4	14.4		6.7	13.6	10.9	3.1	6.8	6.3		Khouvine, '54
4.7	6.3	18.1		7.8	7.4	11.1	5.4	6.1	0.3		Khouvine, '54
9.5–	5.8–	11.1–11.3		7.2–	14.3	9.0–	3.6–	5.0–	5.8–		Pernis, '54b
9.9	6.7			8.3		10.1	4.1	5.7	6.5		Pernis, '54b
8.5–8.7	5.9–6.7	11.3–11.7		8.0–8.7	12.8–13.5	9.0–9.9	4.2–4.4	5.7–5.9	6.3–8.5		Pernis, '54b
7.8–10.2	6.4–8.5	5.8–9.1	2.3–2.9	6.3–8.4	10.1–11.3	7.3–8.5	3.2–3.8	3.9–4.9	5.9–7.1	0.0	Smith, E. L., '55d
13.1	10.2	10.3	4.3	11.0	10.6	10.4	3.2	6.1	8.0		Agren, '52e
9.5	6.7	11.1		7.2	14.3	10.1	3.6	5.0	5.8		Wunderly, '54b
9.9	5.8	11.3		8.3	14.3	9.0	4.1	5.7	6.5		Wunderly, '54b
8.5	6.7	11.7		8.0	13.5	9.9	4.2	5.7	6.3		Wunderly, '54b
8.7	5.9	11.3		8.7	12.8	9.0	4.4	5.9	8.5		Wunderly, '54b
12.5	9.0	11.4	1.7	6.5	17.7	8.6	3.2	6.8	4.8	0.0	Müting, '52b
	5.0	15.5	<0.1	7.7							*Cf.* B & B, '51
6.4	5.7										Neidig, '52
5.5	6.5	10.0	1.8	8.0	11.1	8.5	11.5	7.0	6.5	0.2	Müting, '52
4.4	6.7	9.9	1.9	5.0	14.5	9.4	4.0	6.4	4.5		Müting, '51-'52
	4.7	16.3			4.4	6.8	9.8		2.0		*Cf.* B & B, '51
			0.6								*Cf.* B & B, '51
											Cf. B & B, '51
5.1	5.3										*Cf.* B & B, '51
5.4	4.6	15.9	0.7	8.3	8.3	9.4					*Cf.* B & B, '51
	7.5	16.3	1.9	10.8	7.5	12.3			4.7		van der Linden, '48
6.5	6.9	14.4	1.8	9.3	7.2	10.0	4.3	9.1	4.0		van der Schaaf, '55
5.6	6.2	14.6	1.8	9.1	7.3	9.8	4.4	9.3	4.0		Huisman, '55a
5.6	6.0	14.4	1.3	9.0	8.1	10.4	4.3	8.8	5.2		Dustin, '54a
	5.8		<0.3				4.2	9.5	4.7		*Cf.* B & B, '51
	6.6	16.6	0.5	12.2	7.4	12.4			5.6		van der Linden, '48
5.2	5.8	14.5	0.3	10.6	6.8	10.0	4.3	9.7	4.7		van der Schaaf, '55
4.3	5.3	14.5	0.3	10.4	7.0	10.1	4.3	9.5	4.8		Huisman, '55a
4.9	5.8	14.3	0.0	10.5	7.1	9.6	4.1	9.4	5.3		Rossi-Fanelli, '54a
5.1	6.0	14.9	0.0	11.0	7.4	10.0	4.3	9.8	5.0		Rossi-Fanelli, '55
5.4	5.9	14.8	0.1	10.2	7.6	11.1	4.8	10.5	5.8		Dustin, '54
											Cf. B & B, '51
	3.2										*Cf.* B & B, '51
			0.3	9.2							*Cf.* B & B, '51
5.2	5.4	14.2	0.3	10.4	6.9	9.9	4.3	9.4	4.8		Huisman, '55a
2.2	3.7	9.5	2.7	6.0	12.7	7.3	5.7	5.2	3.7	0	Müting, '51-'52
2.9	5.0	11.5	2.3	7.3	16.4	10.2	2.7	7.0	5.6		Müting, '52b
4.4	5.6	14.4	0.4	10.4	6.9	10.1	4.1	9.5	4.4		Huisman, '55a
5.0	5.6	13.8	0.2	10.0	6.8	10.6	4.6	9.5	4.1		Schroeder, '50b

	Source	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	PHE
STROMA										
Stroma, Embryo	Bovine		13.7	0.81	5.8	2.0	5.3	3.6	1.4	
Stroma, Adult	Bovine		13.8		5.9	2.2	4.1	3.7	1.5	
Stroma	Chicken		11.4		7.3	1.3	6.3			
Stroma	Horse		15.4	0.89	6.3	2.7	4.7	3.7	1.6	
Stroma	Human		13.0	0.88	5.9	2.6	4.7	3.3	1.5	
Stroma, pathological—Polycythema cardiac	Human		13.7		4.0	2.5	3.6			
Stroma, pathological—Polycythema vira	Human		13.1		6.1	2.2	5.0	3.6	1.5	
Stroma	Swine		13.1	0.81	5.6	2.6	4.3	3.4	1.5	
MISCELLANEOUS BLOOD AND URINE PROTEINS										
Angiotonin			M. R.		2	2	1	1	?	1
Bence Jones Protein (urine)	Human	M	16.7		4.9	1.8	6.0	5.2	2.2	2.8
Bence Jones Protein (urine)	Human	CM	16.2		4.8	1.6	5.8	5.4	2.2	2.6
Bence Jones Protein (urine)	Human	CM	16.4		4.7	1.5	5.8	5.5	2.1	2.4
Bence Jones Protein (urine)	Human	CM	16.7		4.7	1.5	5.9	5.4	2.0	2.2
Bence Jones Protein (urine)	Human	CM	16.3		5.0	1.6	5.9	5.0	2.2	2.7
Bence Jones Protein (urine)	Human	X	(16.0)		3.7	0.4	5.5	8.7	1.7	5.9
Bence Jones Protein (urine)	Human	M	16.1		5.7	1.0	4.4	7.3	3.9	2.7
Bence Jones Protein (urine)	Human	C								
Bence Jones Protein (urine), an average	Human	C	16.8	1.17	5.0	1.0	5.0	7.0	1.6	1.5
Bence Jones Protein (urine), Multiple myeloma	Human	CM	(16.0)		3.7–6.7	1.6–2.0	5.9–8.3	4.6–9.4	2.0–3.8	4.5–11.5
Bence Jones Protein (urine), Tumor	Human	M	16.1		5.4	1.2	4.2	4.0	2.5	2.0
Blood Cells	Chicken		15.9					2.6	1.3	4.3
Blood Cells	Human		16.3		3.2	6.3	6.9			
Cerebro-Spinal Fluid		M	(16.0)		5.0	3.3	9.3			8.2
Cerebro-Spinal Fluid, an avg. of 28 individuals					5.3	3.2	9.2			10.1
Erythrocytes, an average			13.90	0.81	6.1	2.4	4.7	3.7	1.5	
Hemocyanin, an average		C	17.5	1.12	6.9	6.0	7.7			
Hemocyanin	Crustacea	C						4.4	5.4	
Hemocyanin	Mollusk	C	(15.6)	1.0	5.5	6.1	8.0	4.6	5.7	
Hemerythrine		C	16.8	1.66	5.4	5.1	8.5	5.9	6.0	
Plasma glycoprotein	Bovine	M	14.95	0.59	5.9	2.1	7.7	2.8		4.6
Plasma mucoprotein	Human	M	10.1	1.62	5.8	2.1	8.0	3.2	2.0	6.2
Plasmapeptide	Calf	M			10.9	4.8	7.9			4.4
Prothrombin	Bovine	CX	14.7		8.9	2.0	6.2	4.8	3.5	5.2
Urine albamin in nephrosis (2 cases)	Human	M	(16.0)		6.1	3.5	12.3			7.5

* B & B '51 refers to Block and Bolling. *The Amino Acid Composition of Proteins and Fo* Springfield, Thomas, 1951.

COMMENTS—Blood and Urine Proteins

Serum Albumins: Although the heterogeneity of the serum albumins was pointed long ago by Sorensen (1930) and others, this fact has been largely forgotten in recent ye Evidence for this heterogeneity has been recently reported by Saifer (1954) using elect phoretic methods.

Globins and Hemoglobins: Van der Linden (1948; 1949) showed that there are differen in the HIS, ISO, THR, MET, PRO, TYR and VAL contents of the newborn, young child (6 mos. to 3 yrs.) and adults. The fact that fetal but not adult hemoglobin contains isol

URINE PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

	SER	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
	1	0	2	1	1	1	2	1	1	2	0	Bumpus, '54
	12.1	8.0	6.5	3.5	6.6	11.8	6.8	5.2	7.9	0		Roberts, E., '50b
	10.9	6.8	5.6	3.2	5.5	12.0	+	4.2	7.2	+		Schreier, '52
	10.6	7.0	5.8	3.0	5.8			4.2	6.9			Schreier, '52
		6.9	5.8	3.2	5.6							Schreier, '52
		7.0	5.7	3.2	5.6							Schreier, '52
	11.0	9.5	9.0	6.4	5.2	15.5	10.6	4.4	5.4	5.9		Dustin, '54b
	16.7	9.5	10.6	4.2	11.0	16.6	6.1	2.3	6.0	6.4	0	Agren, '52c
												Bakay, '51
0			5.4		5.5	7.2	3.8	2.0	5.0	6.9	0	*Cf*. B & B, '51
0	4.3– 7.6	8.3 - 14.2	7.6– 9.6	1.5– 3.2	7.3– 9.3	5.9 10.5	7.0 - 9.1	7.1– 9.7	6.8– 8.1	6.0 7.3		Jirgensons, '52
0	8.1	6.9	8.8	2.6	6.9	13.8	7.8	1.8	3.5	5.6	0	Agren, '52c
0		4.9	9.2	3.2	7.1							*Cf*. B & B, '51
		5.6	12.2	4.9	6.4							Kazmeier, '53
		5.4	12.5	5.0	6.7							Esser, '45
0												*Cf*. B & B, '51
10			10.2		5.8				4.7			*Cf*. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
												Cf. B & B, '51
10		4.4	9.2	2.3	5.4	16.0	10.8	3.5		4.2		Sloane, '52
00	4.0	7.6	8.3	5.0	4.5	17.0	11.8	1.3		3.8		Weimer, '48
80			8.0	4.0	5.2	5.1						*Cf*. B & B, '51
50	6.3	5.1	8.3	3.4	5.3	14.0	10.9	4.9	4.5	6.1		Laki, '54
30		5.8	13.0	1.9	8.0							Schreier, '51

ne has been used by Rossi-Fanelli (1954b) to determine these two proteins in the presice of each other. Schroeder (1950b), on the basis of extensive analyses of the hemoglobins om normal negroes and those with sickle-cell anemia, concludes that the sickle-cell hemoobin contains slightly less LEU and VAL and slightly more SER and THR than hemoobin from normal individuals.

Bence-Jones Proteins: It is now generally agreed that although Bence-Jones proteins differ om individual to individual (*cf*. Putnam, 1954) in a number of physical and chemical roperties, they all contain little or no methionine (Papastamatis, 1949).

	Method	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PH*
AMPHIBIAN									
Frog, spawn mucin	M	8.1	0.95	3.0	1.6	3.5	3.0		2.
ECHINODERM									
Sea Urchin (Paracentrotus lividus)									
Aged Eggs—7 hours, soluble	M	% Molar Sum		4.8	1.4	5.0	2.4		3.
Aged Eggs—7 hours, insoluble	M	"		5.5	1.9	6.1	2.5		3.3
Unfertilized Eggs, soluble	M	"		4.7	1.4	5.1	2.5		3.
Unfertilized Eggs, insoluble	M	"		5.6	1.8	5.7	2.5		3.
50-hour Plutei, soluble	M	"		4.0	1.2	4.5	2.3		3.4
50-hour Plutei, insoluble	M	"		5.9	1.7	6.2	2.4		3.
FISH									
Catfish Roe									
Albumin	C			7.4	1.1	8.2	5.8	0.8	
Globulin	C			7.2	0.8	7.9	4.2	1.3	
Herring Roe	C	12.1		5.4	2.7	7.2	4.4	1.9	5.0
Salmon Roe									
Salmon Roe				7.1	2.8	8.8		1.0	4.9
Salmon Roe	C	12.9		6.4	3.2	7.5	4.7	0.8	5.3
Salmon Roe	M			7.3	2.6	8.8		0.9	4.8
Salmon Roe (King salmon, very immature)	M			7.5	2.3	7.1		0.9	4.4
Salmon Roe (King salmon, very mature	M			7.5	2.5	7.2		0.8	4.2
Salmon Roe (Sockeye, very immature)	M			6.9	2.5	8.8		0.8	4.
Salmon Roe (Sockeye, very mature)	M			7.3	2.5	8.7		1.1	5.0
FOWL									
Duck									
Whole Egg	M								
Egg White	CM			3.4	2.1	5.7		1.2	5.3
Hen									
Whole Egg	CM			6.2	2.1	6.3	3.5	1.1	5.7
Whole Egg, range	M			6.4–6.8	2.7–2.8	6.7–6.9	4.3–4.6	1.8 1.9	5.4
Whole Egg	M			5.7	2.3	7.4	3.2	1.0	5.1
Whole Egg	M	8.11		9.6	2.3	7.9		1.7	7.3
Whole Egg	M	12.16		6.3	2.2	5.7	3.4	1.9	6.
Whole Egg	M			7.0	2.5	7.9			5.4
Whole Egg, an average	CM	12.6	1.66	6.9	2.1	6.6	4.4	1.4	5.
Whole Egg, powder	M			6.7	2.1	6.5		1.7	5.
Whole Egg, powder	X				2.5		5.0		6.
Whole Egg, spray-dried, 10 mos. at room temperature	M			5.7	2.4	7.3			5.
Egg White, spray-dried, 10 mos. at 50°F	M			5.5	2.4	7.3			4.
Egg White	C	(14.5)							
Egg White	C	12.11							
Egg White	M	11.8		5.1	2.7	4.5	4.0	2.0	6.
Egg White, range	M			5.8–6.3	2.6–2.8	6.5–6.7	4.2–4.7	1.7–2.0	6. 6.
Egg White	CM			6.0	2.4	7.2	3.3	1.2	5.
Egg White	M			6.6	2.1	6.2	5.1	1.8	6.
Egg White from chicks fed low lysine and methionine diets	M					6.3			
Isolated Egg White Proteins:									
Albumin	C	15.3	1.70	5.8	2.1	5.6	4.1	1.5	6.
Albumin	M	15.8		6.0	2.4	6.5	2.7		7.
Albumin	M	15.98		6.0	2.1	6.3		1.3	7.
Albumin	M			6.3	2.4	6.8		1.8	6.
Albumin	PX	(15.4)		6.3		7.0	4.4		
Albumin	PX	(15.4)		5.8	1.9		3.2		
Albumin	X				2.5		4.8		6.
Albumin	X	15.4		5.8		7.0	4.4		8.
Avidin	M	14.8	1.03	7.0	1.0	6.7	1.0	5.8	6.
Avidin A	M	(13.8)		7.8	1.0	7.6	1.1	7.0	7.
Conalbumin	M	16.6	1.76	7.3	2.5	5.5	4.4	2.9	5.
Conalbumin, an average	C	15.0		5.8	2.6	7.2	5.0		
Globulin	C	14.4		5.5	1.6	5.7			
Mucin								4.0	
Ovomucoid	M	13.1	2.70	5.4	2.6	7.3	3.9	tr.	3.
Ovomucoid, an average	C	(13.5)					4.2	2.2	5.
Egg Yolk	M			6.7	1.9	5.6	4.8	1.6	4.
Egg Yolk, range	M			7.1–7.4	2.8–2.9	7.1–7.3	4.4 4.6	1.8 1.9	4. 4.
Egg Yolk, an average	CM	14.4	1.53	7.3	1.8	5.5	5.1	1.5	4.
Egg Yolk, from chicks fed low methionine and lysine diet	M					6.5-7.0			
Egg Yolk, dried	M	4.71		8.2	2.7	9.3		1.5	5.
Egg Yolk, dried	M	4.88		7.8	2.6	8.6		1.3	5.

PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

S	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	4.6	4.6	3.8	5.2	4.9	3.4	8.0	7.9	10.4	3.1	7.1		Folkes, '50
	2.3	6.6	6.4	8.7	6.5	8.2	12.8	11.8	4.7	9.0	4.6		Kavanau, '54b
	3.5	6.8	5.0	9.1	5.5	7.4	9.5	8.3	7.9	10.6	4.7		Kavanau, '54b
	2.3	6.5	6.6	8.5	6.2	8.3	12.7	11.4	4.7	9.9	4.6		Kavanau, '54b
	3.6	6.7	5.1	9.1	5.6	7.4	9.1	9.0	8.0	10.1	5.0		Kavanau, '54b
	3.3	6.0	7.1	7.5	5.4	8.2	13.0	11.2	6.8	9.8	4.9		Kavanau, '54b
	3.3	6.2	4.7	8.9	5.1	6.7	9.9	9.2	9.1	10.8	4.7		Kavanau, '54b
													Cf. B & B, '51
													Cf. B & B, '51
	3.0		5.2	10.7	9.9	9.4							*Cf.* B & B, '51
	2.9		5.9	10.0	7.3	7.3							Tarr, '55
	3.5		4.5										*Cf.* B & B, '51 & Block, '49
	3.0		5.7	9.9	7.4	7.2							Seagran, '53
	2.6		6.0	8.2	5.6	6.1							Seagran, '54
	2.2		6.2	9.1	5.5	6.3							Seagran, '54
	3.0		5.8	9.8	7.2	7.1							Seagran, '54
	2.8		5.9	10.7	7.3	7.7							Seagran, '54
	4.5												*Cf.* B & B, '51
	4.6		5.6	7.9	4.7	6.2							Patwardhan, '54
3	3.2		4.9	9.0	6.2	7.0	12.6	10.8	4.0		4.5		Rutgers Univ. Bull., '50
1–	3.1–	7.1–	4.7–	8.3–	6.8–	7.8–	11.9–	5.6–	3.6–				Evans, '49a & '49b
2	3.5	7.9	5.6	8.6	7.1	8.3	13.2	5.8	3.8				
.3	3.0	8.4	4.1	9.2	6.8	6.9	12.6	10.5	3.0				Schieler. '53b
	2.7		5.9	12.6	8.8	7.0							Horn, '49 & '50
.2	3.5		5.2	9.1	5.9	7.9							Frost, '49
	3.3		5.2	8.9	6.5	6.7							Horn, '55
.4	3.8		4.2	9.4	7.5	7.2							*Cf.* B & B, '51
	3.5		5.5	8.2	7.1	8.2							Miller, S., '52
	3.7			10.5	6.2	6.7							Miller, S., '52
	2.9			9.2	7.4	7.1			3.1				Dunn, unpub.
	2.9			9.2	7.4	7.0			3.2				Dunn, unpub.
	3.4– 5.0												Bakay, '51
		8.5	3.7										Neidig, '52
.3	4.1		4.9	9.5	5.8	7.8							Frost, '49
.3–	4.0–	6.3–	4.5–	8.2–	6.8–	8.2–	11.7–	5.8–	3.8–				Evans, '49a & '49b
.6	4.1	7.1	5.2	8.6	7.3	9.0	13.9	6.0	4.0				
.0	4.2		4.9	8.8	6.5	7.5	12.9	11.0	3.8		2.9		Rutgers Univ. Bull., '50
.7	4.0		4.7	9.9	4.6	7.1							Edwards, C.H., '55
	3.8												Milligan, '51
1.9	4.9	8.4	4.0	8.9	7.4	6.1	14.6	8.6	3.8	7.3	4.8	0	*Cf.* B & B, '51
	5.5	8.6	4.0	10.0	7.2	10.4	15.9	9.3	3.2	5.8	3.8		Lewis, J. C., '50
	4.4		4.5	9.4	7.4	7.5							Horn, '49 & '50
	4.1		4.9	7.1	6.5	8.0							Miller, S., '52
1.9	5.7		4.6				16.4	10.4	3.4	8.7	3.7		Caldwell, '54b
	6.6	6.8	3.6	8.9	6.5	8.6	12.1	9.8	3.2	6.6	3.2		De Wael, '54
	4.7			9.9	5.8	8.7							Miller, S., '52
1.7	3.1	5.9	3.5	15.1		7.3	15.6	9.7	3.8	6.2	3.3		Thompson, J., '52
0.5	1.5	4.9	11.3	5.3	5.9	4.5	7.1	10.5	5.0		1.8		Lewis, J. C., '50
0.2– 0.5	1.7	5.2	14.6	5.7	5.9	5.2	7.7	12.3	5.9		1.6		Fraenkel-Conrat, '52a
3.7	2.0	6.1	5.7	6.6	4.8	7.9	11.5	12.8	5.5	4.2	4.7	0	Lewis, J. C., '50
3.5													*Cf.* B & B, '51
													Cf. B & B, '51
													Hill, '49
8.2	1.2	5.1	6.7	6.1	1.7	7.3	7.9	15.9	4.6	2.8	3.3	0	Lewis, J. C., '50
6.2	1.7												*Cf.* B & B, '51
1.6	1.9		5.3	8.5	4.0	5.9							Edwards, C.H., '55
1.6–	2.0–	8.1–	5.0–	8.2–	6.7–	7.1–	11.8–	5.4–	3.3–				Evans, '49a & '49b
1.9	2.6	8.9	6.2	8.6	7.0	7.5	12.2	5.7	3.7				
2.0	2.8	3.6											*Cf.* B & B, '51
	2.4												Milligan, '51
	2.5		6.1	10.9	6.8	7.0							Anderson, D., '53
	2.4		5.9	10.2	6.4	6.7							Anderson, D., '53

	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY	P
Isolated Egg Yolk Proteins:									
Livetin	C	15.1		5.4	1.1	5.1	6.3	1.5	
Phosvitin	CM	11.6		6.6– 8.0	6.6	8.2	0.1	0.8	1
Phosvitin	M	11.9	0	6.4	6.4	7.9	0.1	0.8	0
Phosvitin, dephosphorylated	CM								
Vitellin	M	15.7	1.01	8.4	3.1	7.0	3.9	1.1	4
Turkey	M	1.72							
REPTILE									
Turtle	C			7.6	2.2	7.0	4.9	1.1	4.

G PROTEINS (Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.1	2.4			11.0			7.0	3.1		6.4	2.3		*Cf.* B & B, '51
.0	0.4			1.4	<1	<1	4.7	6.1	2.2		1.4		Mecham, '49
.0	0.4	44.0	1.9	1.3	0.7	1.5	4.6	5.9	2.2	2.0			Lewis, J.C., '50
		45.8	tr.										Mecham, '49
.5	2.9	11.4	4.8	8.8	5.4	6.3	11.2	8.3	2.9	4.1	4.5	0	Lewis, J.C., '50
	3.6												*Cf.* B & B, '51
.5	2.5		4.4										*Cf.* B & B, '51

	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	P
GASTROINTESTINAL									
Enterogastrone, "EP-10" (Picric acid purified)	M	(15.0)		7.0	3.5	10.2	3.3		3
Enterogastrone, "ET-10" (Tannic acid purified)	M	(15.0)		7.8	3.8	9.6	3.2		3
Secretin, an average value	C	14.1	0.80	7.3	4.0	9.9	0	2.0	
PANCREATIC									
Insulin	CMX	15.3		3.0	5.2	2.8	12.8		8
Insulin	X			3.0	5.3	2.9	11.6		8.
Insulin, bovine	PX	M.R.		1	1.9	1.0	3.8		3.
Insulin, bovine	calc'd			3.0	5.4	2.6	12.6		8
Insulin, bovine	X	15.54		3.2	5.6	2.7	12.9	0	8.
Insulin, bovine	X	15.54		3.2	5.4	2.6	12.6	0	8.
Insulin, bovine	X	(15.4,		2.1	3.2	2.2	8.6		
Insulin, bovine	X			3.3	6.0	2.7	12.7	0	8.
Insulin, sheep	X	15.54		3.0	5.3	2.7	13.4	0	9.
Insulin, swine	X	15.54		2.8	5.5	2.6	12.9	0	8.
Insulin, swine	X	(15.4)		2.1	3.9	2.3	8.6		
Strepogenin, from insulin	X	M.R.			1				
Strepogenin, from insulin, impure	X	M.R.			1				
Glucoprotein, prepared from bovine pancreas		14.0		6.0	2.0	7.0	tr.	2.7	11.
PITUITARY, ANTERIOR									
Gonadotropic Hormones:									
Follicle-Stimulating Hormone (FSH, Gonadotropin), sheep	C			1.0	1.9	7.4	7.0	1.5	
Follicle-Stimulating Hormone (FSH, Gonadotropin), sheep	CM	15.1	1.40	5.6	3.9	11.8	4.0		6.
Follicle-Stimulating Hormone (FSH, Gonadotropin)	CM			5.3	3.7	11.1	3.8		5.
Lactogenic Hormone (Prolactin), sheep	CM	15.9	1.80	8.6	4.5	5.3	4.7		4.
Metabolic Hormones:									
Adrenocorticotropic Hormone (ACTH, Corticotropin), sheep	CM	15.6	2.40	8.9	1.3	5.1	2.5		4.
Adrenocorticotropic Hormone (ACTH, Corticotropin), sheep	M	(16.0)		8.7	1.3	5.0	2.4		4.
Adrenocorticotropic Hormone (ACTH, Corticotropin)	M	(16.0)		8.7	1.3	5.0	2.4		4.
Adrenocorticotropic Hormone (ACTH, Corticotropin)	M	(16.0)		9.0	1.3	4.6	2.4		4.
Adrenocorticotropic Hormone (ACTH, Corticotropin)	X	(16.0)		10.3	1.3	5.3	3.0		4.
Adrenocorticotropic Hormone (ACTH, Corticotropin)	X	12.95		8.2	0.5 ?	4.5	5.1		6.
Adrenocorticotropic Hormone (ACTH, Corticotropin)	X	16.04	4.62	3.6	0.7	2.8	3.6	0	5.
Adrenocorticotropic Hormone ("Acthar," Armour)				8.9		7.1	3.2		3.
Adrenocorticotropic Hormone ("Cortrophine," Organon)				8.5		7.3	3.1		3.
Adrenocorticotropic Hormone ("Cibacthen," Ciba)				8.0		7.5	3.4		3.
α-Corticotropin, sheep	PX	M.R.		3	1	4	2	1	3
β-Corticotropin (peptide ?)	X	M.R.		3	1	4	2	1	3
β-Corticotropin (or corticotropin B)	X	17.6	>0.50	12.2	2.8	10.4	6.3	6.6	4.
Corticotropin A, hog		M.R.		2.0	1.0	3.0	1.0	1.0	2.
Corticotropin, Hypo-active fraction	X	9.2		4.8	4.4		2.1	6.6	
Corticotropin, Urinary	X	6.40		5.6	6.4			10.5	
Growth Hormone (GH, Somatotropin), ox	CM	15.7	1.30	9.3	2.7	7.3	5.3	0.8	8.1
Growth Hormone (GH, Somato tropin), an average value	M	(16.0)		9.1	2.7	7.1	5.2	0.9	7.
PITUITARY, POSTERIOR									
Melanophore-expanding Hormone, hog	PX	M.R.		1.9	1.5	3.7	1.7	2.1	1.
Oxytocin	C	(16.0)		>0.8			14.2		
Oxytocin	X	M.R.					1		
Oxytocin, bovine	X	M.R.					0.9		
Oxytocin, swine	X	M.R.					0.9		
Oxytocin	X	M.R.					1		
Vasopressin	C	(16.0)		12.3			11.9		
Vasopressin, swine		M.R.				1.0	0.7		1.
Vasopressin, bovine	X	M.R.		1.0			0.7		1.
Vasopressin	X	M.R.		1.0			1		1
Vasopressin	X	19.5		8.9	0	0	8.5	0	8.5

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.1	2.3		4.8	7.3	4.7	5.4	14.8				3.3		Millen, '52
2	2.3		4.5	7.7	4.5	5.3	15.3				3.7		Millen, '52
5	3.6	2.2		6.4					3.1	5.3			*Cf.* B & B, '51
.0	0	5.4	2.3	15.3	1.7	7.7	20.2	5.7	5.0	4.8	2.1		Acher, '50
0		5.6	2.7	12.2	3.2	9.1	18.7	6.8	4.7	4.5	2.2		Mills, G.L., '52
8		2.3	1.0	7.1		4.6	6.7	2.9	4.2	3.0	1.0		Levy, '54b
6		5.5	2.1	13.7	2.3	10.2	17.9	7.0	5.2	4.6	2.0		Tristram, '53
6	0	5.4	2.1	13.9	1.6	10.0	17.8	6.9	5.3	4.7	2.2	0	Harfenist, '52-'53
9	0	5.6	2.2	13.8	1.4	9.9	18.2	7.2	5.7	5.0	2.2	0	Harfenist, '53
.7		6.5				9.9			8.2		1.8		Lens, '52
3	0	5.9	2.3	14.2	2.8	8.8	20.6	7.3	4.9	4.6	2.7		Grassmann, '55
.5	0	3.9	2.1	14.2	1.6	10.1	18.4	7.2	6.4	4.8	2.3	0	Harfenist, '52-'53
0	0	5.2	3.8	14.0	3.6	7.7	18.5	6.9	5.3	3.4	2.2	0	Harfenist, '52-'53
7		5.6				8.1			7.4		2.5		Lens, '52
		1		1		1	1						Merrifield, '55
		1		2		1	1			1.0			Merrifield, '55
r.	0	6.3	0	8.0		11.0	8.0	8.6	2.0	10.0	9.0	0	Mohnike, '53
													Cf. B & B, '51
7	1.1	3.4	5.0	9.8	3.5	6.1	14.2	9.9	2.2		5.5		Li, '50-'51
3	1.0		4.7	9.2	3.3	5.8	13.4	9.3			5.2		Li, '52a
1	3.6	6.5	4.8	12.5	7.2	5.9	14.1	11.6	4.0		6.2		Li, '50-'51
4	2.0	6.2	3.3	8.0	3.2	3.5	16.0	6.9	8.2		8.4		Li, '50-'51
.6	1.9	6.0	3.2	7.8	3.1	3.4	15.6	6.7	8.0		8.2		Li, '52b
.2	1.9	6.0	3.2	7.8	3.1	3.4	15.6	6.7	8.0		8.2		Mendenhall, '53
.6	1.2		2.1	7.2	3.4	3.3	16.1	6.3			7.7		Mendenhall, '53
.2	(0.9)	6.7	3.6	7.4	3.0	3.5	15.0	8.3	8.7	7.0	9.6		Mendenhall, '53
.5	0.2 ?	4.2	0 ?	23.5	4.7	11.1	9.0	13.3	35.2	2.0	4.9	0 ?	DeBarbieri, '51c
.9	0	4.8	3.6	7.4	4.7	4.2	19.0	6.4	11.9	6.7	8.4	0	Block, '52
		4.2	2.3	11.3		5.3	9.1	17.5	5.1	7.1	7.0		Pernis, '54a
.7	2.2	4.5	2.3	12.3		5.9	9.1	17.5	4.8	7.7	6.3		Pernis, '54a
.5	2.0	4.1	2.5	10.7		5.3	9.1	17.9	5.1	7.5	6.6		Pernis, '54a
	1	3	0	1	0	3	5	2	3	3	4	0	Li, '54a & Levy '55
	1	2	0	2	0	3	5	2	3	3	4	0	Bell, '54
	2.3	4.1	0	0.0	0	6.1	7.4	4.8	4.0	1.7	5.3		?
	1.0	1.0	0	2.0	0	2.0	4.0	1.0	2.0	2.0	3.0		White, '53
													DeBarbieri, '52a
.6	0 ?												DeBarbieri, '52b
.6	3.0	5.8	6.2	12.4	4.0	4.0	13.3	9.2	3.9		3.5		Li, '50-'51 & '54b
.0				12.1	4.0	3.9	13.0	9.0	3.8				*Cf.* B & B, '51
.1	0.9	1.7		0.2		0.3	4.0	3.5	2.3	0.2	3.4		Benfey, '55
													Cf. B & B, '51
.3				1	1		1	1	1		1		Tuppy, '53
				1.0	1.0		1.1	1.1	1.1		1.0		Pierce, '52
.9				1.0	1.0		1.2	1.1	1.2		1.1		Pierce, '52
.7				1	1		1	1	1		1		Pierce, '50ab
													Cf. B & B, '51
.0							1.1	1.0	1.0		1.0		Popenoe, '52
.7							1.1	1.0	1.0		1.0		Popenoe, '53
.7							1	1	1		1		Turner, '51a
.9		0.6	0	0.4		0.3	8.1	6.9	4.2	0	6.4		Turner, '51b

	Meth-od	Nitro-gen	Sulfur	ARG	HIS	LYS	TYR	TRY	P
THYROID									
Thyroglobulin, Normal:									
Bovine	C	uncorr.	0.60*				3.5	2.3	
Dog	C	uncorr.	0.40*				3.5	2.3	
Human	C	(16.0)		7.4	1.8	5.8	3.3	2.0	
Swine	C		0.40*				2.6	2.1	
Swine	C	15.6		8.2	6.9	3.9	5.5	2.2	
Swine	C	uncorr.	0.35-0.72*	12.7	2.2	3.4	3.1	2.1	6
Thyroglobulin, Pathological:									
Adenomatose	C	(16.0)		7.4	1.7	5.6	3.2	2.1	
Colloid Goiter	C	(16.0)		7.4	1.8	5.4	3.4	2.2	
Exophthalmic Goiter	C	(16.0)		7.4	1.7	4.7	3.1	2.1	
Hyperthyroid (bovine)	C		0.01*				5.5	2.3	
Hyperthyroid (swine)	C		0.04*				3.6	2.1	

* Indicates iodine values

COMMENTS—HORMONES

Insulin: Insulin is split into two peptide chains by oxidation. The amino acid sequen in the phenylalanine chain is identical in the insulins obtained from cattle, pig, and she pancreas. The glycine chains of the three species of insulin are identical except for positi 8, 9, and 10. These vary in the following manner:

Position	8 9 10
Cattle	ALA•SER•VAL
Pig	THR•SER•ISO
Sheep	ALA•GLY•VAL

(Brown, H., 1955: Springall, 1955).

Insulin, Oxytocin and Vasopressin: "It is interesting that the ring (formed by S closure of two cysteine residues) is the same size as the similar disulphide ring found oxytocin and vasopressin, which suggests that it may have a possible structural or biologi significance" (Ryle, 1955).

Adrenocorticotropic Hormone: Li (1955) has described the structure of α-corticotrop as: ARG, 3; HIS, 1; LYS, 4; TYR, 2; TRY, 1; PHE, 3; MET, 4; SER, 3; LEU, 1; VAL, GLU, 4; ASP, 2; GLY, 4; ALA, 3; PRO, 4. These values differ slightly from those fou from amino acid analyses only. It is interesting to note that the hormone derived from she

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
											Thyroxine	Diiodotyrosine	
3.5											0.3		Derrien, '49
													Derrien, '49
		10.8											*Cf.* B & B, '51
3.6											0.3		Derrien, '49
1.6													*Cf.* B & B, '51
3.6	1.3	10.8		12.8		1.5			3.7	7.4	0.2	0.5	Derrien, '49
											0.1	0.3	*Cf.* B & B, '51
											0.0	0.1	*Cf.* B & B, '51
											0.2	0.5	
1.6											0		Derrien, '49
2.2											0		Derrien, '49

ituitary contains •ALA•SER• in positions 31 and 32, while that derived from hogs as LEU•ALA• in these positions.

Oxytocin and Vasopressin: These two physiologically important octapeptides differ only the two amino acids, the isoleucine and leucine of oxytocin being replaced by phenylanine and a basic amino acid in vasopressin. The oxytocins from both beef and hog pituiries are identical, while beef vasopressin contains 1 mol. of arginine which is replaced y 1 mol. of lysine in vasopressin from hogs (Pierce, 1952; Popenoe, 1952, 1953; Tuppy, 953).

Analytical Errors: The structures and consequently the exact amino acid composition of e hormones, insulin, ACTH, oxytocin and vasopressin, are known. However, study of e reported amino acid values point to the rather large errors for many of the amino acids r these highly purified, carbohydrate-free proteins and peptides. It is therefore not surrising that the analytical values for less purified proteins and for the crude proteins in ods are subject to even greater analytical difficulties. The errors in the analyses of oxytocin nd vasopressin are apparent because every amino acid is in a 1:1 ratio with all the rest. the case of ACTH, Mendenhall (1953) has reported analyses on the *same* preparation y two independent laboratories using the microbiological method (Shankman and Hier) nd his own using ion exchange column chromatography. It will be seen that the range of eviations in the amino acid values is from 0 to 111 per cent, with an average deviation of 4 per cent.

	Source	*Meth-od*	*Nitro-gen*	*Sul-fur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PI*
WHOLE MILK										
Colostrum										
Colostrum, 0-hour	cow	M			5.0	2.7	7.7		1.8	
Colostrum	cow	M	(16.0)		4.3	2.5	6.9			4.
Colostrum	cow	M			4.2	2.4	7.9			4.
Colostrum, 24-hour	cow	M			4.9	2.8	7.8		1.7	
Colostrum, mixed colostrum and transitional	human	M			3.4	1.9	5.4		1.4	3.
Colostrum	ewe	M			3.9	2.5	7.8			4.
Colostrum	sow	M			6.1	2.2	6.7			4.
Mature Milk										
Dried	bitch	PX			3.5	3.6	4.8	2.0		3.
Dried	cow	CM	6.57							
Dried	cow	M			3.1	1.2	7.1	4.4	1.2	6.
Dried	cow	M			4.4	2.7	7.5		1.6	5.
Dried	cow	MPX			3.5	2.8	9.4	5.0	1.1	5.
Dried	cow	M	(16.0)		3.1	2.7	7.4			4.
Dried	cow	M			3.4	2.6	8.3			4.
Dried	cow	X			3.7	3.2	7.9	5.8		5.
Dried	cow	X			4.0	3.6	8.1	4.5		4.
Dried, an average	cow	CM			3.5	2.5	7.8	5.6	1.4	5.
Dried	ewe	M			3.2	2.7	8.4			4.
Dried	ewe	M			4.5	2.9	7.6		1.5	5.
Dried	g'n'a pig	M			7.1	3.4	9.9	6.4	2.5	5.
Dried	human	M			2.8	1.9	5.9	4.2	1.8	3.
Dried	human	M			3.6	2.0	5.6		1.6	3.
Dried	human	M			2.8	1.5	5.9	9.9	1.2	4.
Dried	human	PX			4.2	2.7	8.2	2.4	1.3	3.
Dried	human	PX			3.7	2.2	7.2	3.3		3.
Dried	human	X	14.6		3.3	2.3	6.8	4.9	2.0	4.
Dried, an average	human	C	15.2	1.6	4.7	2.5	6.9	5.2	2.0	5.
Dried	mare	M			6.9	3.1	6.9		1.3	5.
Dried	sow	M			5.9	2.2	7.5			3.
Dried	sow	M			5.7	2.8	6.9		1.3	4.
Dried	sow	PX	13.8		5.1		4.7	5.5		
Dried	cow	M								
Evaporated (Borden)	cow	M			3.2	2.7	7.7			4.
Evaporated (Wilson)	cow	M			3.1	2.7	7.2			4.
Terminal Milk										
Terminal	cow	M			4.1	2.8	7.7		1.4	
Terminal, 60-day milk	cow	M			4.2	2.9	8.1		1.5	
SKIMMILK										
An average	cow	CM	6.4							
Dried	cow				3.2	2.5	8.4	4.1	1.2	4.
Powder, spray dried	cow				3.0	3.0	8.7			5.
Powder, roller dried	cow				2.6	2.7	8.0			5.
CASEIN										
Casein	cow	C	13.3							
Casein	cow	C	(15.4)						3.1	
Casein	cow	C	15.37							
Casein	cow	C	15.75			2.6				
Casein	cow	CM	(16.0)		4.2	3.0	8.0		1.2	5.
Casein	cow	CM	15.6		4.2	3.2	8.4	6.5	1.2	5.
Casein	cow	CPX	15.6							
Casein	cow	CM			3.9	3.0	8.1	5.2	1.0	5.
Casein	cow	M	13.22		3.9	3.4	7.0	5.5	1.4	5.
Casein	cow	M			3.8	3.0	8.3			6.
Casein	cow	M	(15.4)		3.5	3.1	8.3	7.5	1.5	5.
Casein	cow	M	16.1		3.8	2.6	8.2		1.2	4.
Casein	cow	M	15.7							
Casein	cow	M			4.1	3.0	7.9		1.3	5.
Casein	cow	M	(15.4)							
Casein (vitamin-free)	cow	M			7.3	3.1	8.0			5.
Casein, an average	cow	CM			5.4	4.0	9.6	9.0	2.5	6.
Casein	cow	M	14.3		3.9	3.1	8.7	5.6	1.2	5.
Casein	cow	M			4.3	3.3	9.8	5.6	1.4	5.
Casein	cow	PX			3.8	1.7	7.1			
Casein	cow	PX	(14.0)		3.8		8.2	5.7		6.
Casein	cow	PX			4.9	3.7	8.6	6.1		6.
Casein	cow	X				1.7				5.
Casein	cow	X	13.5		3.4	2.9	8.1	5.4	1.1	4.
Casein	cow	X	(16.0)		3.6	3.2	8.5	5.4	1.4	5.
Casein (from raw milk)	cow	C	11.7		3.5	3.3	8.2	6.9	1.6	6.
Casein (from fresh pasteurized milk)	cow	C	11.5		3.2	3.3	8.4	6.7	1.6	6.
Casein (from cured pasteurized milk)	cow	C	11.6		3.6	3.0	8.5	6.9	1.6	6.
Casein	ewe	C	(15.4)							
Casein (from colostrum)	human	C							2.4	
Casein	human	C	14.6		5.2	1.7	6.7	5.9	1.4	6.

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.8		7.6	8.6	5.4	8.5							Duncan, '52
			2.0	7.3	4.3	8.0							Sarkar, '49b
	1.9		6.8	8.9	4.6	7.6							Kuiken, '49
	1.9		7.6	8.9	6.0	8.3							Duncan, '52
	1.2		4.1	7.1	4.9	5.2							Miller, S., '50
	1.9		6.8	9.5	4.7	7.9							Kuiken, '49
	1.2		5.3	9.3	4.0	6.4							Beacom, '51
4.5	2.7	4.5	4.3	12.1	6.8	6.7	18.0	9.2	1.5	3.5	14.3	1.0	Block, unpub.
					5.8								Horn, '49
0.4 ?	3.3	6.7	4.1	11.7 ?	11.7 ?	7.1	26.3	6.0	2.4		7.2		Kirimura, '52
	2.2		5.6	8.8	6.1	7.2							Sarkar, '53
0.8	2.1	5.7	4.8	10.1	5.3	6.6	22.3	8.1	1.9	3.6	9.8		Bigwood, '53b
			4.8	8.5	5.7	6.7							Sarkar, '49b
	2.6		4.3	9.0	5.7	7.0							Kuiken, '49
0.8	2.7	5.5	5.1	9.8	5.6	6.1	21.4	8.3	1.8	3.5	9.4	0	Bigwood, '54
	1.8	5.3	3.6	12.5		5.2	22.0	7.4	2.0		10.2		Kraut, '55
0.9	2.7		4.4	9.8	6.2	6.6							*Cf.* B & B, '51
	2.5		4.3	9.6	5.5	7.4							Kuiken, '49
	2.6		5.2	9.5	6.8	7.8							Sarkar, '53
	2.7		4.8	10.6	6.0	8.4							Nelson, '51
1.5	1.7	4.5	4.4	9.2	5.0	5.0	18.3	8.3	2.2	3.5	8.0		Bigwood, '53b
	0.9		4.2	7.6	4.8	5.8							Miller, S., '52
0.6	2.1	2.8	4.1	10.8 ?	10.8 ?	5.3	19.0	5.5	2.0		8.0		Kirimura, '52
0.9	1.1	4.0	4.3	8.7	4.3	5.1	12.8	8.4	2.2	3.7	7.0		Cheung, '53
1.1	1.0	4.2	4.2	9.0	3.9	4.8	13.6	8.1	2.2	3.5	6.6		Cheung, '53
2.5	2.2	5.2	5.2	10.5	5.5	5.7	22.6	9.8	2.4	4.1	8.8		
2.8	2.2		5.2	9.2	6.7	8.1							*Cf.* B & B, '51
	2.1		4.7	9.4	7.0	8.1							Sarkar, '53
	1.4		3.6	8.2	4.3	5.1							Beacom, '51
	2.0		5.1	8.3	5.9	6.2							Sarkar, '53
1.6	1.2	5.8	3.7			4.3	11.2	5.1	2.0				Block, unpub.
			4.8	11.8	6.5	6.2							*Cf.* B & B, '51
	2.2			9.3	7.1	6.8	20.0		2.2				*Cf.* B & B, '51
	2.3			10.4	7.5	7.3	21.0		2.2				*Cf.* B & B, '51
	2.4		5.5	8.9	7.0	7.6							Duncan, '52
	2.2		4.8	9.8	6.8	7.5							Duncan, '52
		4.3	4.6	10.7	6.5	6.1	21.5		2.3				*Cf.* B & B, '51
	2.0		4.3	9.9	7.0	6.3							Williams, H. ., '55
	2.3			10.2	7.5	7.4	22.0		2.3				Dunn, unpub.
	2.5			9.8	7.5	7.0	19.0		2.2				Dunn, unpub.
	3.5												Bakay, '51
	5.4												Spisni, '54
				10.9		6.4							Schweigert, '44
													Vickery, '44
0.2	3.4		4.0	9.8	6.9	6.0							Berkhout, '48-'49
0.3	2.9	6.5	5.0	9.4	6.3	7.4	23.0	7.3	2.8	3.1	11.6		Gordon, '49ab
									2.1	3.3	10.9	<0.1	Gordon, '50
0.4	3.3		4.5	10.0	6.4	7.4	22.4	7.3	2.0		13.4		Rutgers, Univ. Bull., '50
0.4	3.4		4.6	9.8	5.8	7.4							Frost, '49
	3.0		3.6	9.7	6.2	7.0	23.0		2.1				Dunn, unpub.
0.7	2.7	6.8	6.0	11.0	8.6	7.4	22.3	5.9			15.9		Fang, '50
	2.6		4.8	9.2	5.6	6.9							Horn, '50
									2.1				McCoy, '53a
	3.4		4.3	8.6	6.6	8.2							Miller, S., '52
									2.0				Shankman, '47
	3.3		4.3	10.0	6.0	7.3	23.0	3.6	2.0				Murphy, '50
0.7	4.9	7.5	4.9	16.8	8.5	8.1	27.7	8.9	2.1	3.7	14.0	0	*Cf.* B & B, '51
	2.3	7.0	4.4	10.8	6.6	7.3	27.5	5.9	2.2		12.8		Tamura, '52
	3.5		4.9	10.1	6.9	9.6							Williams, H.H., '55
													Waite, '53
0.0	2.7	5.4	4.4	15.2		6.9	22.6	6.9	2.3	3.1	12.9		Thompson, J.R., '51
0.6	3.4	5.5	4.9	10.9	7.0	6.8	25.1	6.4	2.4	3.1			Krishnamurthy, '55
	3.2			10.5	5.9	7.9							Miller, S., '52
0.5	3.0	5.8	4.8	9.1	5.9	6.6	21.5	7.6	2.1	3.1	10.1		Grassmann, '55
0.3	3.4	6.7	4.2	14.8		6.5	22.5	6.7	2.1	3.2	12.3		Kraut, '55
0.4	3.5		3.6	9.0	7.1	7.8							*Cf.* B & B, '51
0.2	3.5		3.7	8.9	7.3	7.5							*Cf.* B & B, '51
0.5	3.6		3.6	8.7	7.4	7.7							*Cf.* B & B, '51
	2.6												Spisni, '54
			5.0	7.9	5.4	6.9							*Cf.* B & B, '51
		5.8	4.6	10.7	6.1	6.8	22.4	4.9		2.1	9.5	0	*Cf.* B & B, '51

	Source	Meth-od	Nitro-gen	Sulfur	ARG	HIS	LYS	TYR	TRY
Casein	rabbit	MPX	(16.0)				7.3		
α-casein	cow	CPX	15.5						
α-casein	cow	CM	15.5		4.4	3.0	9.2	8.4	1.7
β-casein	cow	CPX	15.4						
β-casein	cow	CM	15.4		3.5	3.2	6.8	3.3	0.7
γ-casein	cow	CPX	(15.4)						
γ-casein	cow	CM	15.4		2.0	3.8	6.4	3.8	1.2
WHEY PROTEINS									
Whole whey	rabbit	MPX	(16.0)				5.3		
Lactalbumin	cow	C ?	(15.1)						1.4
Lactalbumin	cow	M			3.3	2.3	10.5		
Lactalbumin	cow	M	15.39						
Lactalbumin	cow	M	15.4		3.6	1.6	11.2		1.8
Lactalbumin	cow	X			2.1	1.2	7.5	2.0	1.1
Lactalbumin	cow	PX							
Lactalbumin, an average	cow	CM	14.8	1.43	3.5	2.1	9.6	4.4	2.3
Lactalbumin, an average of 10 commercial samples	cow	C	14.07	1.3					2.1
Lactalbumin	rabbit	MPX	(16.0)						
Lactalbumin	sheep	C ?	(15.1)						2.0
Crystalline Whey Proteins:									
α-Lactalbumin	cow	PX			1.5	3.9;3.2	10.2	4.8	
α-Lactalbumin	cow	X	15.86	1.96	1.2	2.9	11.6	5.4	7.1
β-Lactoglobulin	cow	C	15.6						
β-Lactoglobulin	cow	C	(15.5)						
β-Lactoglobulin	cow	M	15.6		3.0	1.7	11.5	4.0	
β-Lactoglobulin	cow	X			3.1	1.7	12.1	3.9	2.7
β-Lactoglobulin	cow	PX	(15.6)		2.7	1.0	11.6	4.0	2.0
β-Lactoglobulin	cow	PX	(15.6)		2.9	1.7	13.3	3.6	2.1
β-Lactoglobulin	cow	PX			2.7	1.7	11.8	4.4	
β-Lactoglobulin	cow	PX			3.1	2.1	12.1	3.9	2.5
β-Lactoglobulin	cow	PX, X							
β-Lactoglobulin, an average	cow	CMX	15.6	1.69	3.0	1.8	11.1	4.1	2.0
β-Lactoglobulin	rabbit	MPX							
MISCELLANEOUS MILK PROTEINS									
Carniferrin	cow	PX			0.2		3.0	0.9	
Fat-globule membrane	cow	M	12.36		6.5	2.2	7.9	4.1	1.2
Fat-globule membrane	cow	M	12.36		9.3	2.7	9.7	4.1	1.2
Fat-globule membrane, from nonhomogenized milk	cow	M	12.20		9.2	4.0	7.8		2.3
Fat-globule membrane, from homogenized milk	cow	M	13.30		7.1	3.9	9.1		1.4

COMMENTS—MILK PROTEINS

The relatively large quantity of nonprotein amino acids (approximately one-half of t total nitrogen) in breast milk as compared to cows' milk (approximately 1/20 of the tot nitrogen) has been pointed out by Block and others (*cf.* Block, 1950; Block and Bollin 1951). The production of amino acids, peptides, and amines during the ripening of cheese discussed by Block (1951b). Aschaffenburg (1955) has made the interesting observation th different breeds of cows show different kinds of β-lactoglobulin, *i.e.*, a cow may produc either pure β_1-lactoglobulin, β_2-lactoglobulin or a mixture of β_1 and β_2. This observatio

K PROTEINS (Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

S	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
						8.0			1.2				Campbell, '52
									2.3	3.9	7.7		Gordon, '50
	2.6	6.5	5.1	8.2	6.6	6.5	23.2	8.7	2.9	3.8	8.5		Gordon, '49ab
									1.6	2.1	15.7		Gordon, '50
	3.5	7.1	5.3	12.1	5.7	10.6	24.1	5.1	2.5	1.8	16.6		Gordon, '49ab
									1.5	2.4	17.7		Gordon, '50
	4.3	5.7	4.6	12.5	4.6	10.9	23.8	4.2	1.6	2.4	17.7	0 ?	Gordon, '53b
						6.4							Campbell, '52
	0.7												Spisni, '54
	2.3			13.3	8.2	4.0	18.0		2.6				Dunn, unpub.
					6.2								Horn, '49
	1.7		5.6	13.4	6.4	6.0							Horn, '50
	1.0		4.9	9.1	5.5	4.5							Williams,H.H.,'55
		4.1	5.0			5.8	19.6	11.8	1.4	7.3	5.3		Brown, G.L., '53
	2.7	5.5	5.4	12.2	7.3	6.4	15.4	10.4	3.5	6.6	4.0	0	*Cf.* B & B, '51
	2.6												Hess, '48
									4.5				Campbell, '52
	1.1												Spisni, 54
	0.9	4.4	5.2	9.8	6.8	4.4	12.2	18.8 ;24.1	3.3	1.9	1.7	0	Block, '55
	1.0	4.8	5.6	11.6	6.9	4.8	13.0	18.8	3.2	2.2	1.5		Gordon, '53a, '54, '55
											4.9	0.1	Troll, '53ab
		4.0	5.2				18.8	10.7	1.6				Keston, '52
	3.2	3.9	5.2	15.6	7.5	6.4	19.9	11.6	1.4	6.0	5.1	0	Lewis, J.C., '50
	3.7	5.3	4.9	16.1	9.3	5.8	20.8	11.5	1.7	7.0	5.9		Spies, '51b
	3.0	3.5	4.7	11.1	5.2	4.6	20.2	11.5	1.5	6.5	5.0	0	McFarren, '52
	3.1	4.0	5.0	16.1	5.7	5.7	20.2	11.8	1.6	7.1	5.2		Roland, '54
													Slotta, '52
	3.6	4.7	5.8	15.4	8.4	5.8	17.8	14.0	1.5	7.0	5.0	0	Block, '55
		4.1	5.0			6.2	20.5	11.1	1.0		5.4		Brown, G.L., '53
	3.1	4.6	5.6	15.9	8.2	6.0	20.2	11.0	1.5	7.1	5.0	0	*Cf.* B & B, '51
									3.7				Campbell, '52
	3.3	3.3	6.1			5.0	11.8	9.0	1.1	2.5			Block, '53
	2.6	4.1	8.3	11.6	4.5	7.0		6.0	3.9		6.0		Hare, '52
	2.7	6.2	9.4	11.5	5.7	8.0		6.3	4.0		6.2		Hare, '52
	2.7		7.9	11.4	7.4	7.4	16.8		4.9				Brunner, '53
	2.6		6.8	11.1	7.2	6.6	21.4		2.0				Brunner, '53

lains some of the previous discrepancies in the literature. β_1- and β_2-lactoglobulins are arated by liquid or paper electrophoresis.

ven in the recent literature there are reports which indicate that the authors believe that protein composition of milk of ruminants may be influenced by the amino acid composition of the feed. This is obviously impossible as the ruminant does not derive its amino ls from the feed but from the tissue proteins of the microorganisms of the rumen. That he nitrogenous constituents of the feed are converted by the rumen microorganisms into ir tissue proteins and the animal uses these tissue proteins as its own source of amino ls. Any changes in the protein composition of the milk is, then solely due to the genetic e-up of the animal.

	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY
CRUSTACEA								
Crab	M			5.1	1.9	4.8	4.5	1.0
Lobster (Bombay)	M	14.23		7.2	1.2	17.6	0.8	0.2
Prawn (Bombay)	M	15.53		8.3	1.6	18.5	1.0	0.4
Prawn (Bombay)	PX			8.8	3.6	6.6	3.5	
Shrimp	M			8.5	1.8	8.1		1.0
FISH								
Boi (Bombay)	M	15.7		6.6	1.9		1.6	0.4
Bonito (Japan)	M	17.4		9.4	6.2	10.0	1.6	0.7
Burbot (Michigan)	M					11.9		
Carp (Chile)	C						3.3	0.9
Carp (Michigan)	M					10.1		
Cod, meal	M			5.7	2.8	9.5	3.2	0.8
Red Cod *(Sebastodes spp.)*				4.3	1.6	14.4	0.4	
Flounder (Chile)	C						3.1	0.7
Haddock	M			5.5	2.0	9.9		1.1
Halwa (Bombay)	M	14.18		3.7	1.3		1.3	0.7
Herring	M			5.1	4.7	8.7		1.0
Herring, meal	M			8.1	2.8	9.5	2.9	0.9
Herring, meal	M	12.0		8.3	2.6	7.9	3.2	1.0
Herring (Michigan)	M					10.6		
Herring *(Clupea pallasii)*				5.9	2.0	8.2	2.2	0.7
Liqua Fish	M			5.5	1.2	6.1	2.4	0.7
Mackeral	M			5.0	5.6	7.8		1.0
Mackeral, Horse	M	16.1		9.3	3.0	10.0	1.7	0.4
Menhaden, meal	M			6.5	2.7	8.7	2.6	1.1
Mushi (Bombay)	M	14.03		5.2	1.7	16.5	1.5	0.8
Pakat (Bombay)	M	14.58		5.2	1.7	19.5	1.4	0.6
Pike (Alaska)	M					10.8		
Pollock (Alaska)	M			7.4	2.2	8.7		0.9
Pomfret (Bombay)	M	15.02		6.1	1.8		1.3	0.6
Rawas (Bombay)	M	14.67		7.4	1.7	19.8	1.6	1.0
Red Fish, meal	M			6.7	2.1	10.8	2.8	1.0
Salmon, canned, average of 3 varieties				5.7	1.9	6.8		1.1
Sardine	M			5.2	2.1	8.2		0.9
Sardine, meal	C			5.4	2.6	9.8		1.1
Sardine, canned				5.1	4.7	8.4		1.0
Shark, meal	CM							
Smelt (Michigan)	M					11.1		
Stockfish (Chile)	C						2.8	0.6
Suckers, (Michigan)	M					10.8		
Swordfish (Atlantic)				9.1	3.3	7.2		1.3
Trout	M					12.3		
Tuna	M			5.3	6.0	8.7		
Tuna	M			5.8	2.4	9.7		1.2
Tuna	M			5.2	5-7	8.4		0.8
Tuna, canned, 3 varieties, average				5.3	5.7	8.3		1.0
Turbot (Japan)	M	17.6		8.7	2.6	9.1	1.2	0.6
Whitefish (Michigan)	M					10.9		
Miscellaneous fish preparations:								
Fish protein	M			19.7	2.0	9.4	6.4	1.7
Fish, meal	C	11.7	0.9	6.0	3.1	8.8	3.0	1.0
Fish, meal	M			6.6	2.2	8.8	2.8	0.8
Fish solubles, spray-dried	M			4.8	1.2	7.8	1.9	0.6
FOWL								
Chicken		(16.0)		5.6	1.6	5.9	2.4	0.9
Chicken, Gizzard	M			9.5	1.7	7.6	3.0	1.0
Various fowl: Chicken, Duck, Turkey, range	M			5.7–6.1	2.2–2.3	8.3–8.8		
MAMMAL								
Whole Muscle								
Beef, a range	X			5.3–5.5	3.7–3.9	9.2–9.4	3.8 4.0	
Beef	CM			6.4	3.3	8.6	3.0	1.0
Beef	M							
Beef	M			6.6	2.9	7.9		0.9
Beef	M			5.4	3.8	9.3	4.1	1.1
Beef	M	15.65		7.5	4.4	10.0	2.5	1.9
Beef	M			6.8	3.2	8.0		1.3
Beef, canned	M			6.3	3.6	9.6		
Beef	M			6.1	3.6	8.7		1.0
Beef	M							
Beef	M	(16.0)		7.9	2.0	10.0		1.3
Beef	M			6.6	2.9	8.4		1.1
Beef	M	(16.0)			3.3	12.1	4.0	1.2
Beef, average of various cuts	M			6.7	3.1	8.5	3.4	1.1
Beef, fresh, aged and cooked samples	M			Reference only				
Beef, tongue	M					7.6		
Beef, tongue	M							

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.6		3.3	4.6	3.6	5.2							Williams, H.H.,'55
.5	2.2		5.3	11.3	4.3	2.9							Master, '54
.4	4.6		4.6	14.3	5.6	4.1							Master '54
.7										5.8			Airan, '54
	2.6		4.1	8.0	5.0	5.0	15.5	9.3	6.8				Dunn, '49d
.5	4.4		7.1	11.3	5.9	5.5							Master, '54
	2.1	2.8	2.4	4.6	3.7	7.8	6.4	6.8	5.7		4.7		Sugimura, '54
	3.0		4.5	7.9	4.9	4.8							Ingalls, '50
.3	3.8												Saul Sas, '50
	2.7		3.7	6.7	4.5	4.5							Ingalls, '50
.3	2.9	4.9	6.1	10.2	7.6	6.7	20.2	12.2	5.8	5.1	6.0		Agren, '51
	2.6		5.1	11.4	6.8	5.0							Tarr, '55
	3.8												Saul Sas, '50
.0	2.9		4.5	7.9		6.3							Proctor, '50
r.	1.8		4.1	6.2	3.4	2.9							Master, '54
	3.0		4.5	7.2	5.1	5.6	13.4	8.8	5.6				Dunn, '49d
.9	3.1	5.6	4.3	7.8	5.6	7.0	13.4	11.2	7.4	7.0	5.8		Agren, '51
													Cf. B & B, '51
	2.5		4.8	7.6	5.1	5.7							Ingalls, '50
	2.2		4.5	8.0	6.4	5.1							Tarr, '55
	2.2		2.8	5.5	5.2	4.0							Williams, H.H.,'55
	2.8		4.5	7.7	4.5	5.3	12.0	8.0	6.4				Dunn, '49d
	2.0	2.5	2.2	4.1	3.2	7.4	6.3	6.5	4.8		5.0		Sugimura, '54
	2.7		4.7	8.1	7.3	5.8							Williams, H.H.,'55
.9	2.0		5.4	7.9	5.4	4.3							Master, '54
.0	1.6		4.6	12.5	5.2	3.5							Master, '54
	2.8		4.3	7.3	4.5	5.0							Ingalls, '50
	3.0		5.6	7.4	5.2	4.8							Landgraf, '53
.3	3.0		6.9	10.7	5.2	5.0							Master, '54
.3	3.7		8.1	8.8	7.3	6.5							Master, '54
	2.7		4.2	7.4	6.3	5.2							Williams, H.H.,'55
	3.0		4.4	7.3	4.9	5.6							Tarr, '55
	2.8		4.7	7.0	4.4	5.1	12.8	8.3	5.1				Dunn, '49d
													Cf. B & B, '51
	2.8		4.3	7.2	4.6	5.2	13.0	9.0					Tarr, '55
	1.4			6.4	5.2	4.3							*Cf.* B & B, '51
	2.8		4.3	7.8	4.8	5.4							Ingalls, '50
.2	3.3												Saul Sas, '50
	2.6		3.8	7.3	4.5	4.8							Ingalls, '50
	3.8		2.6	7.8	7.5	4.7							Tarr, '55
	2.6		4.5	7.4	5.1	5.7							Ingalls, '50
	2.8		4.4	7.3	4.9	5.4	14.0	8.4	4.5				Murphy, '50
	2.7		4.7	5.6	5.4	6.1							Anderson, '53
	2.8		4.5	7.3	5.0	5.6	12.9	8.7	4.6				Dunn, '49d
	2.8		4.5	7.2	4.7	5.1	13.0	8.7					Tarr, '55
	1.9	2.4	2.0	3.6	2.6	6.3	6.0	5.8	4.3		4.2		Sugimura, '54
	2.9		4.1	7.8	5.1	5.7							Ingalls, '50
.1	4.2		6.1	18.9		10.2							Baertich, '50
.3	2.8	2.9	4.6	8.5	6.5	5.9							*Cf.* B & B, '51
	3.0		4.5	7.5	7.0	5.4							Williams, '55
	2.2		2.5	7.5	4.1	4.7							Williams, '55
.9	2.5		4.1	9.4	2.6	2.6							Guaita, '49
.0	2.2												
	2.3– 2.7		3.5– 4.5	7.3– 7.8	4.6– 5.2	4.7– 4.9	16-18	9.7– 11.0	4.6– 6.7				Dunn, '49c
.3– .5	2.8– 3.1	4.1– 4.5	4.8	8.1– 8.7	5.2	4.8– 5.5	15.8– 16.2	9.7– 9.9	4.6– 6.1	6.1– 6.3	3.0– 4.1		Bigwood, '53a
.3	2.7		4.5	7.8	5.2	5.1	14.6	9.3	5.6		5.1		Rutgers Univ. Bull., '50
.9– .3		4.3– 5.2							4.5– 6.7	6.2– 6.8			Alexander, '53
	2.0		4.3	8.3	4.8	5.2							Anderson, '53
.3	2.9	4.5	4.7	8.2	5.2	5.0	15.9	9.8	4.5	6.4	3.6		Bigwood, '53b
.2	3.0		5.6	9.2	4.8	6.0							Frost, '49
	2.7		4.5	7.0	5.5	5.8							Miller, S., '52
	2.7		4.6	8.4	5.4	5.4	15.1	9.1	5.8				Murphy, '50
	2.7		4.5	7.8	5.0	5.2							Neilands, '49
				7.7		5.0							Schweigert, '44
	2.8		5.8	9.4	6.5	5.9							Horn, '49 & '50
	2.3		4.0	8.4	5.1	5.7							Schweigert, '54
.9	2.4		4.0	7.7	2.8	6.0							Clark, '55
.4	2.2	4.0	4.0	8.6	5.3	5.9	14.8	8.8	7.3		5.6		Greenwood, '51
													Ginger, '54
	2.3		4.7	7.7	5.7	5.0							*Cf.* B & B, '51
				7.7		4.9							Schweigert, '44

	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY
Human, range	CPX			2.7 5.5	2.5 3.9	7.0 10.1	1.9 3.7	1.5 2.7
Human	M			6.1	2.3	9.6	4.0	1.1
Human	M			6.1	2.7	8.8	3.1	0.8
Lamb	M							
Lamb	M	2.01		6.8	2.5	9.4		1.0
Lamb	M							
Lamb	M			6.7	2.6	7.7		1.3
Lamb	M			6.9	2.7	7.7		1.3
Lamb, average: chop, loin spareribs, loin, shoulder	M						3.1	
Rabbit	M			5.6	2.3	8.8		
Rabbit, average	CPX			2.7	2.2	7.2	2.8	
Rat	M			5.8	1.7	7.7		
Rat, an average	CPX			4.5	4.0	9.0	2.8	1.8
Swine								
Pork	M							
Pork	M	2.36		6.7	3.1	9.6		1.1
Pork	M			6.3	3.3	8.5		0.9
Pork	M							
Pork	M			6.4	3.2	7.8		1.4
Pork	M			4.5	2.9	8.1	3.7	1.3
Chitterlings (intestine)	M			16.4	2.0	7.8	2.7	1.1
Fatback, raw	M			9.7	0.9	8.1	1.4	0.2
Fatback, cooked	M			11.0	1.4	8.5	1.9	0.5
Ham	M			6.0	3.0	8.5		0.8
Tongue	M			5.8	2.3	7.8		
Tongue	M			6.1	2.0	10.2	3.1	1.4
Veal, *see also* Beef								
Veal	X			6.5	3.6	8.6	3.4	
Veal	M							
Veal	M	2.17		7.1	2.4	9.2		1.1
Veal	M			6.4	3.6	8.6	3.2	1.1
Veal	M							
Whale		(16.0)		12.4	1.3		3.5	1.1
Whale, vacuum-dried meat	M	12.5				9.4		0.9
Various meat preparations:								
Meat, meal	M			5.8	2.4	5.4	2.9	1.0
Meat, scraps	C		1.1	6.2	2.8	5.6	3.2	0.7
Meat, scraps	M			6.8	2.1	7.8	1.8	0.6
Meat, mammalian, range (cat, dog, rat rabbit, mouse)	M			5.4 6.3	1.9 2.4	7.7– 9.0		
Meat, mammalian, an average	C			6.5	2.1	8.0	3.5	1.2
Skeletal muscle	M						3.5	
Tankage, an average	C		0.8	5.8	2.6	6.1	2.9	0.8
MOLLUSK								
Squid *(Ommastrephes spp.*, Japan)	M	16.3		11.4	2.5	8.9	1.6	0.0
Squid *(Loligo spp.*, Japan)	M	14.6		12.6	2.3	10.0	1.5	0.3
REPTILE								
Iguana, an average value (Mexico)	M	12.63		5.6	3.2	9.4		1.1
ISOLATED MUSCLE PROTEINS:								
Actin				7.2	2.4	8.7	4.1	2.1
Actin	X	16.6		6.3	2.8	7.3	5.6	2.0
Myoglobin, whale	X	17.0	0.4	2.1	10.5	15.0	2.2	3.4
Myoglobin, human	PX			2.5	7.8	19.1	2.2	3.4
L-Meromyosin	X	16.7		8.5	2.8	11.6	2.0	1.2
N-Meromyosin	X	16.7		4.8	1.7	11.5	3.6	0.6
Myosin	X	16.7		6.8	2.2	11.9	3.1	0.8
Myosin, ling cod *(Ophiodon elongatus)*				4.8	2.7	15.0	2.7	0.9
Tropomyesin	X	16.7		7.0	0.8	15.4	2.6	0.0

COMMENTS—MUSCLE PROTEINS

The amino acid composition of muscle proteins appears to be practically the same a

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
2.2–4.8	1.8–2.3	6.3–8.5	5.7–8.3	9.5–11.0	3.2–4.5	6.7–8.0	12.0–15.8	5.0–6.3	2.2–4.5	6.0–7.1	6.5–8.2	3.5–6.5	Müting, '54
2.1	2.2	5.0	3.7	7.1	6.0	5.7	14.9	5.9	5.2	9.2	4.8		Bocobo, '52
1.4	2.4	4.8	3.8	7.4	8.5	4.5	10.5	4.4	5.8	6.8	6.1		Reynolds, '53
1.0		4.7–5.0							4.2–4.6	6.1–6.2			Alexander, '53
	2.2		4.7	9.1	5.1	5.5							Anderson, '53
				8.1		4.8							Schweigert, '44
	2.4		4.8	7.1	4.6	5.0							Schweigert, '49b
	2.3		4.9	7.4	4.8	5.0							Schweigert, '54
1.4		3.8					14.3	8.0	6.8		4.8		Schweigert, '51
	2.7		4.8	7.9	5.2	4.9	17.0	10.0	4.7				Murphy, '50
2.5	1.9	8.2	7.5	9.5	2.4	7.5	15.1	6.2	1.9	5.7	7.0	4.2	Müting, '54
	2.5		4.2	7.9	6.8	6.7							Solomon, '51
2.8	2.1	8.6	7.7	10.2	3.5	7.1	13.5	5.0	3.8	6.5	4.0		Müting, '54
1.0		5.2							4.1	6.1			Alexander, '53
	2.5		4.3	8.6	5.3	5.7							Anderson, '53
	2.3		4.7	7.8	4.8	5.2							Neilands, '49
				7.3		5.3							Schweigert, '44
	2.5		5.1	7.5	4.9	5.0							Schweigert, '54
1.2	2.5		4.5	7.0	5.3	5.2							Edwards, C.H., '55
1.3	2.3		4.6	5.3	3.6	5.4							Edwards, C.H., '55
1.1	1.4		3.6	9.4	2.8	4.3							Edwards, C.H., '55
0.7	1.6		3.0	4.6	2.5	5.3							Edwards, C.H., '55
	2.4		3.2	7.7	4.8	5.1	14.4	8.4	6.2				Dunn, '49d
	2.1		3.8	7.3	4.1	4.8	12.6	9.3	7.4				Camien, '49
1.5	1.9		4.9	7.4	5.0	5.6							Edwards, C.H.,'55
1.3	2.4	4.0	4.7	7.7	4.6	4.8	14.6	9.1	4.2	5.6	3.5		Bigwood, '53a
1.0		4.7							4.7	6.2			Alexander, '53
	2.1		4.6	8.6	5.1	5.5							Anderson, '53
1.3	2.4	3.6	4.5	7.7	4.5	4.5	14.5	9.1	4.1	5.4	3.6		Bigwood, '53b
				7.3		5.3							Schweigert, '44
	3.3		4.0										Schulze, '49
0.9	2.6												Carpenter, '55a
0.4	2.8	5.6	3.0	9.6	2.7	5.9	11.5	4.3	1.8	8.4	6.3		Agren, '49a
1.2	1.9	3.7	3.7	8.0	3.4	6.1							*Cf.* B & B, '51
	1.4		3.3	6.5	4.0	5.1							Williams, H.H.,'55
	2.3–2.7		3.9–5.0	7.0–8.1	4.3–5.3	4.5–5.1	16.0–18.0	9.7–11.0	4.3–7.1				Dunn, '49c
1.1	3.2	5.6	4.2	8.0	5.8	5.1	17.2	5.8		6.2	4.3		*Cf.* B & B, '51
							16.5	10.0			4.0		Violante, '52
0.9	1.7	3.3	3.3	10.0	3.1	5.7	9.4						*Cf.* B & B, '51
	1.4	2.5	2.3	4.7	3.2	6.8	6.9	5.5	5.9		5.0		Sugimura, '54
	1.1	3.2	2.3	5.3	3.6	7.7	8.2	8.1	5.9		4.3		Sugimura, '54
	2.6		7.5	9.7	9.2	5.3							Suárez, '54
1.1	4.6						15.5	9.1					Ivanov, '50
1.3	4.3	5.6	6.7	7.9	7.2	4.7	14.2	10.4	4.8	6.1	4.9		Kominz, '54
0.0	1.8	2.5	4.0	16.9		3.5	16.4	6.0	3.8	8.0	2.2		Schmid, '49ab
0.0	2.7	4.4	2.9	13.7	5.3	4.6	16.2	8.3	6.1	5.8	5.4		Rossi-Fanelli, '55
0.6	2.0	3.7	4.3	10.7	4.4	4.4	24.5	9.8	1.7	6.5	0.9		Kominz, '54
1.3	2.7	4.3	5.6	9.8	5.3	5.1	19.5	11.3	3.2	6.2	3.2		Kominz, '54
1.0	3.1	4.1	4.7	9.9	5.3	4.7	21.9	10.9	2.8	6.7	2.4		Kominz, '54
	2.3		5.8	10.2	7.7	6.0							Tarr, '55
0.8	2.3	4.0	3.2	11.9	3.0	4.3	29.7	11.4	0.9	9.4	0		Kominz, '54

e different classes of animals. Some of the differences shown in the table are due to ana-
tical errors or, in the case of histidine, to the quantities of nonprotein histidine (from
rnosine and anserine) present in the preparation.

	Source	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	PHE
COLLAGENS AND GELATINS										
Bysuss Fibers										
from *Pinna nobilis*			14.70		8.3				6.60	
from *Mytulis edulls* and *Anna nobilis*		PX			5.8		6.2	10.3		
Bone										
Bone	Human	X								
Bone	Ox	X								
Cartilage	Bovine	X								
Collagen, unspecified sources										
Collagen, an average		CX	18.30		7.6	0.7	3.6	1.0	0	3.6
Collagen		PX & X						0.0		
Dentin										
Normal		CM			7.4	0.8	3.3	1.1	0	1.9
Normal		C	16.15							
Normal	Human	C	16.09		7.4	0.7	2.4-3.3	1.1	0	1.9
Carious		CM			7.2	0.8	3.3	1.3	0	1.9
Dura Mater		PX	(16.00)		7.1		4.5	5.4		2.5
Gelatin										
Gelatin		C	uncorr.							
Gelatin		C	17.60							
Gelatin		C	(16.00)							
Gelatin		M			9.0	0.7	4.4			2.2
Gelatin		M	18.32		8.0	0.7	3.9		0	2.0
Gelatin		M	17.60							
Gelatin		M				0.6				1.8
Gelatin		X				0.7				2.3
Gelatin, an average value		CM		0.40	7.8	0.8	4.8	0.6	0.01	2.0
Gelatin, from calfskin		M	17.40		5.9	0.6	4.8	0.1		1.7
Gelatin, from fish scales		M	17.60		8.1	0.9	4.5	0.6		2.0
Gelatin, from skin or bone		X								
Isinglass (Ichthyocol)										
Isinglass		C	17.83		8.6	0.7	3.6			
Isinglass		X								
Isinglass, an avg. value		C	18.20	0.63	8.2	1.0	3.8	0	0	1.6
Procollagen										
Procollagen		C	(16.00)					0	0	
Procollagen	Bovine	X	17.00		8.5	0.8	4.6	1.0	0	2.5
Procollagen	Ox		17.04		9.2	2.9	4.6	0	0	2.3
Scales, Fish	Herring	CM	10.62		7.2	2.2	3.3	1.7	0.17	2.8
Skin (Hide)										
Beefskin		X								
Beefskin		X	16.15		7.9	0.9	3.8	0.8	0	2.5
Calfskin		X	18.60		7.6	0.7	3.9	1.2	0	2.2
Calfskin		X								
Calfskin, citrate-extracted material		X	17.70		8.0	0.4	3.4	0.5		2.0
Calfskin, citrate-extracted material		PX	17.70		7.5	0.3	3.2	0.5		1.8
Cattle Hide		M	17.80		7.8	0.6	4.8	0.8		2.1
Cattle Hide			(17.70)				3.8			
Fishskin		M	18.40		8.2	0.8	4.9	0.7		2.1
Ox Hide		PX	18.60		7.1	0.6	3.4	0.9		2.0
Porkskin		X								
Tendon										
Tendon		C	17.25		8.6	0.8	3.6			
Tendon		M	16.90		7.6	0.9	3.3	0.9		2.4
Kangaroo Tail		C	17.45		8.3	0.8	3.7			
Rat Tail		PX & X			6.4	0.3		0.8		
ELASTIN										
Elastin	Cattle	M	17.10		0.8	0.1	0.4	1.5		4.7
Elastin		PX	16.85		1.2	0.1	0.5	1.3		5.9
Elastin				0.16	1.0	0.04	0.5	1.4	0	3.8
Young "light" elastin		M			0.9	0.1	0.4	3.0	0.10	3.3
Young "heavy" elastin		M			2.2	0.4	0.9	3.6	0.30	3.7
α-Elastin, a degradation product		PX	(16.85)		1.1	0.03	0.5	1.5		
β-Elastin, a degradation product		PX	(16.85)		0.9	0.05	0.5	1.0		4.4

* From Ox.

TRUCTURE PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

ET	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*HO-LYS*	*Reference*
	6.6	3.9						8.0	10.0				Lucas,'55
0.7	6.8	6.7	3.2	2.3	6.2	8.9	7.0	14.7	11.5	6.7			Fitton-Jackson, '53
												0.7	Hamilton, '55b
												0.9-1.2	Hamilton, '55b
												1.4	Hamilton, '55b
.7	3.2	2.0	4.8		2.9		3.2	22.9	8.1	13.6			*Cf.* B & B, '51
.7	3.5	2.4			4.1	14.1	6.4	24.0	10.5	9.6	14.8		Brown, G.L., '53
.5	3.5	3.1	3.6	1.3	3.0	10.6	5.6	22.5	8.5	16.3	13.1		Hess, '54
	6.7	3.1											Neidig, '52
.5	3.1*	1.9*						10.0*	8.5*		13.0		Losee, '51
.5	3.1	3.4	3.9	1.3	2.9	10.6	5.5	22.2	8.4	16.0	12.6	1.0	Hess, '54
.0	3.5	2.2	6.2		3.8	11.0	6.2	20.0	10.0	14.0			Wunderly, '54b
												0.7	Bergström, '51
	4.2	2.2											Neidig, '52
										14.0	12.8		Troll, '53a-'53b
.9		2.1	3.3	1.5	2.8	11.4	6.3	26.4					Dunn, '49b
.7		1.8	2.9	1.1	2.5								Horn, '49-'50
								23.8					McCoy, '53a
.7			2.5	1.6	2.6								Miller, S., '52
.6			3.2	1.6	2.3								Miller, S., '52
.9	3.4	1.7	3.5	1.4	2.7	7.8	4.9	15.7	7.9	14.8	13.3	0.8	*Cf.* B & B, '51
.8	2.7	2.0	2.9	1.7	2.4	11.1	6.3	24.8	8.0	12.9	13.1		Neuman, '49-'50a
.0	3.2	3.0	2.3	1.1	2.0	10.4	5.9	24.9		12.8			Neuman, '49 and Block, '49c
												0.9-1.1	Hamilton, '55b
													Salo, '49-'50
												0.7	Hamilton, '55b
.4	3.6	2.8	3.3	2.6	2.0	7.2	4.1			15.6			*Cf.* B & B, '51
								25.0		25			Orokhovich, '50
.7	3.9	2.0	4.1	2.4	2.8	9.9	5.6	21.5	9.3	13.2			Grassmann, '55
.7						11.0	5.2	28.0	9.5	20			Chernikov, '49
.8	7.5	3.1				7.2		20.6		8.6			*Cf.* B & B, '51
												0.9	Hamilton, '55b
.6	3.7	1.6	2.8	1.4	2.2	9.2	4.9	21.5	8.6	13.5			Grassmann, '55
.7	2.9	2.1	4.8		2.9	9.7	5.4	22.5	8.2	13.0	10.5	1.1	Bowes, J.W., '53
												1.2	Hamilton, '55b
.8	4.1	2.1	3.1	1.4	2.5	11.9	6.0	26.4	10.8	11.5	12.7	0.9	Bowes, J.H., '53
.7	3.9	2.0	2.9	1.3	2.0	10.0	5.5	23.6	9.0	11.8	12.3	1.2	Bowes, J.H., '55
.8	2.5	2.0	2.9	2.0	2.4	10.3	6.3	24.7	7.9	14.2			Neumann, '49
													Sale, '50
.0	5.1	3.0	3.2	1.5	2.2	10.0	6.2	25.7	8.0	11.9	9.1		Neuman, '49-'50a
.8	3.7	1.9	3.2	1.6	2.1	9.6	6.0	22.9	8.9	12.4	11.0	1.0	Bowes, '55
												0.6	Hamilton, '55b
													Salo, '49-'50
.9	2.4		3.3	1.8	2.7	9.9	6.5			10.0			*Cf.* B & B, '51
													Salo, '49-'50
.7	3.7	2.3			3.8	10.9	5.9	26.0	10.5	15.0	12.9		Brown, G.L., '53
.03	0.8	0.9	8.1	3.7	16.3	2.0	0.6	28.0	17.7	15.9	1.0		Neuman, '49-'50a
tr.	0.9	1.1	8.6	3.7	16.5	2.4	1.0	25.5	21.1	11.6	1.5		Partridge, '55
.4	1.8		7.0	3.5	13.2	2.8	0.3	25.7		13.6	1.9		*Cf.* B & B, '51
.1	0.3	0.9	6.8	3.2	17.4	3.1	0.6	22.4	24.0	13.3			Lansing, '51
.6	0.8	1.5	7.1	3.5	15.4	5.1	0.7	18.3	22.0	12.1			Lansing, '51
tr.	0.9	1.1	7.9	2.9	16.1	2.5	0.6	22.0	23.3	11.7	1.4		Partridge, '55
tr.	0.8	0.9	7.7	3.6	14.7	2.0	1.0	27.6	19.8	13.1			Partridge, '55

	Source	Method	Nitrogen	Thyroxine	Iodine	ARG	HIS	LYS	TYR
GORGONINS AND SPONGINS									
Gorgonins									
Antipathes myriophylla		C	(16.0)		2.18	3.1	12.5	12.8	4.5
Antipathes subpinnata		C	(16.0)		1.29	3.1	15.4	11.0	3.5
Cirripathes anguina		C	(16.0)		2.90	3.3	15.4	6.9	5.9
Cirripathes spiralis		C	(16.0)		4.07	3.3	12.6	6.6	6.7
Ellisella andamnanensis		C	(16.0)		0.70				0.5
Ellisella elongata		C	(16.0)		1.80	8.6	1.9	4.3	3.2
Ellisella paraplexaurides		C	(16.0)		1.61	8.4	3.2	4.2	3.0
Eunicella ctenocelloides		C	(16.0)	0.18	8.90	8.2	2.7	2.6	4.6
Eunicella verrucosa, var. stricta		C	(16.0)	0.12	6.78	8.9	2.2	6.3	4.9
Eunicella verrucosa, var. typica		C	(16.0)	0.20	9.30	6.4	2.8	4.5	5.3
Euplexaura maghrebensis		C	(16.0)	0	0.23	7.8	1.5	5.9	1.1
Euplexaura pseudobuttikofferi		C	(16.0)	0	0.49	9.1	2.0	6.8	2.4
Euplexaura purpuroviolacea		C	(16.0)	0	0.06				1.3
Funiculina quadrangularis		C	(16.0)		0.10	8.5	0.9	3.4	0.6
Gerardia savaglia		C	(16.0)		0.80	4.1	13.1	6.0	7.8
Gorgonia adamsii		C	(16.0)		0.54	7.5	1.1	7.2	3.2
Gorgonia coarciate		C	(16.0)		0.30				2.4
Gorgonia crevauxi		C	(16.0)		0.34				2.5
Gorgonia rutila		C	(16.0)		0.08				1.6
Gorgonia stenobrochis		C	(16.0)		0.17				1.3
Gorgonia ventalina		C	(16.0)		0.22	6.6	0	6.1	1.4
Leptogorgia chevalieri		C	(16.0)	0	0.13				1.0
Leptogorgia patechizans		C	(16.0)		0.60	8.7	1.5	9.9	2.5
Leptogorgia sarmentosa		C	(16.0)		0.16	7.0	1.3		2.2
Muricoides chuni		C			1.95	9.7	1.1	5.3	2.5
Paramuricea placomus		C			1.98	7.6	1.7	10.9	4.7
Plexaura kukenthalii		C		0	2.45	6.9	0.9	7.9	5.5
Rhipidigorgia elegans		C	(16.0)		0.08	4.5	0.8	1.9	1.7
Rhipidigorgia flagellum		C	(16.0)		0.62	6.6	0	6.9	8.0
Scirpearia erythraca		C	(16.0)		0.60				0.5
Scirpearia flagellum		C	(16.0)		1.80	8.4	3.3	3.8	3.0
Scirpearia ramosa		C	(16.0)		0.36				1.7
An average value		C	14.8			6.7	1.0	3.6	15.3
Spongins									
Aplysina crassa		C	(16.0)		2.35	3.0	0.7	9.7	2.1
Aplysina fistularis		C	(16.0)		1.30				2.9
Aplysina holda		C	(16.0)		0.47				2.3
Callyspongia vaginalis		C	(16.0)		0.39				1.9
Euspongia officinalis		C	(16.0)		0.51	5.8	0.3	5.2	0.3
Haliclona viridis		C	(16.0)		0.41				2.9
Hircinia variabilis		C	(16.0)		0.57				1.9
Ianthella sp		C	(16.0)		tr.				4.8
Luffaria cauliformis		C	(16.0)		0.47				2.1
Spongilla lacustris		C	(16.0)		0.20				1.3
Stebospongia plicifera		C	(16.0)		0.12				1.7
Syphoncalia pruvoti		C	(16.0)		0.20	4.9	0	3.5	0.7
Uruguaya corraloides		C	(16.0)		0.50				2.9
Verongia fistularis		C	(16.0)		0.50	3.3	1.1	8.7	4.2
An average value		C	13.5			5.4		4.0	1.0
KERATINS				*Sulfur*					
Baleen	Whale	C	14.1	3.9		7.0	1.6	4.3	5.7
Beak		C							
Beak	Emu	C	13.2						10.7
Beak	Iguana	C	15.5						7.0
Black Bill	Goose	C	10.6						13.0
Excrescence	Pelican	C	11.0	1.8		6.9	1.4	4.0	6.5
Egg, Casing									
Egg, Casing	Hen	C	16.4	3.6		7.7	1.3	2.8	3.0
Egg, Casing	Herring	C	14.2			7.1	2.4	6.3	
Egg, Casing	Python	C	14.1			5.8		1.4	
Egg, Casing	Salmon	C	14.5			6.1	1.4	3.7	5.3
Egg, Casing	Silkworm	C	14.4			5.1	1.8	4.1	
Egg, Membrane									
Inner	Duck	C	% Dry Wt.						
Outer	Duck	C	% Dry Wt.						
Whole	Duck	C	% Dry Wt.						
Inner	Hen	C	% Dry Wt.						
Outer	Hen	C	% Dry Wt.						
Whole	Hen	C	% Dry Wt.						
Inner	Turkey	C	% Dry Wt.						
Outer	Turkey	C	% Dry Wt.						
Whole	Turkey	C	% Dry Wt.						
Whole	Salmon	C	13.7						4.1

* Monoiodotyrosine

† Diiodotyrosine

(Calculated as g. of amino acid to 16.0 g. N *or* molar ratio)

PHE	*CYS*	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*MIT* *	*DIT* †	*Reference*
	2.5	0.3	3.5							14.6				0.5	1.4	Roche, '51a
	2.5	0.4	4.3							13.0				0.5	1.0	Roche, '51a
	1.5	0.4	2.5							16.4				4.4	2.1	Roche, '51a
	1.7	0.9	2.3							12.4				7.1	2.2	Roche, '51a
																Roche, '51a
	3.4	0.4	3.4							14.8				2.5	1.2	Roche, '51a
	3.6		4.0							12.7				2.3	2.0	Roche, '51a
	2.1		2.9							15.1				8.8	9.5	Roche, '51a
	3.0		2.2							18.8				9.3	5.1	Roche, '51a
	2.6		1.6							16.7				7.1	12.7	Roche, '51a
	2.8		5.6							13.8				0	tr.	Roche, '51a
	2.1		2.4							15.0				tr.	tr.	Roche, '51a
														0	0	Roche, '48
	1.9	0.3	8.1							20.0				0	tr.	Roche, '51a
	2.2		2.5							23.0				tr.	1.4	Roche, '51a
	2.8		3.7							14.0				tr.	0.7	Roche, '51a
																Roche, '51a
																Roche, '51a
																Roche, '51a
																Roche, '51a
	3.6		3.0							14.0				tr.	tr.	Roche, '51a
														0	tr.	Roche, '48
			4.7							16.3				tr.	tr.	Roche, '51a
			4.4							15.6				tr.	0	Roche, '51a
	3.1		5.0							14.3				tr.	2.0	Roche, '51a
	3.6		3.9							15.8				tr.	1.0	Roche, '51a
	2.8		3.5							12.6				2.3	3.2	Roche, '51a
														tr.	tr.	Roche, '51a
			9.3							14.0				tr.	tr.	Roche, '51a
																Roche, '51a
	3.6	0.4	3.5							15.0				tr.	1.3	Roche, '51a
																Roche, '51a
7.1	9.6															*Cf.* B & B, '51
	1.8	0	3.0							9.5				tr.	4.2	Roche, '51d
															1.4	Roche, '51d
	0.3	0.3	6.2		1.7		1.3			7.7				tr.	tr.	Roche, '51d
															0.8	Roche, '51d
	1.5	0	8.0		0		2.2			15.8				tr.	0.8	Roche, '51d
															0.8	Roche, '51d
																Roche, '51d
																Roche, '51d
																Roche, '51d
																Roche, '51d
																Roche, '51d
	1.0	0.4	4.7							12.0						Roche, '51d
																Roche, '51d
	2.3		6.3							10.4				tr.	tr.	Roche, '51d
4.1	3.4							18.6	4.6	15.2		6.0				*Cf.* B & B, '51
																Cf. B & B, '51
3.2	10.8															
																Cf. B & B, '51
3.4	4.7															*Cf.* B & B, '51
4.9	6.8															*Cf.* B & B, '51
7.2	6.3															*Cf.* B & B, '51
4.9	4.6															
																Cf. B & B, '51
	9.9	5.0	2.2					13.4	10.2			3.7				*Cf.* B & B, '51
																Cf. B & B, '51
																Cf. B & B, '51
	2.0	2.8														*Cf.* B & B, '51
													1.1			Neuman, '50b
													0.7			Neuman, '50b
													0.9			Neuman, '50b
													1.1			Neuman, '50b
													0.9			Neuman, '50b
													1.0			Neuman, '50b
													0.9			Neuman, '50b
													0.6			Neuman, '50b
													0.7			Neuman, '50b
																Cf. B & B, '51
4.8																

	Source	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	T
KERATINS									
Feathers									
Whole:									
Whole	Duck	C	15.9	3.00					
Whole, (Down)	Goose	X	16.47		6.3	0.3	1.4	3.6	
Whole, (Feather meal)	Chicken	PX			7.3	1.7	2.6	2.3	
Whole, an average value	Chicken	CM	15.6	2.70	7.3	0.6	1.6	2.3	
Whole, (Leghorn)	Chicken	M		2.67	6.8	0.3	1.0	2.0	
Whole	Gull	C	(16.0)						
Feather Parts:									
Barbs	Turkey	X	16.45		6.3	0.4	1.2	2.3	
Barbs and Barbules	Chicken	M	16.5	2.63	6.5	0.3	0.9	1.6	
Calamus	Chicken	M	15.9	2.22	6.1	0.6	1.6	2.8	
Calamus	Turkey	X	17.39		6.2	0.5	0.9	3.7	
Medulla	Turkey	X	17.01		6.2	0.7	1.2	3.6	
Rachis	Turkey	X	16.8		5.4	0.3	0.8	2.8	
Shafts	Chicken	M	16.7	2.59	5.9	0.3	0.7	1.6	
Barbis and Rachis	Various	X	(16.0)					4.4	
Hair									
White, an average of 3	Dog	C							
White, an average of 3	Guinea Pig	C							
White, an average of 3	Rabbit	C							
Gray	Guinea Pig	C							
Very light brown	Dog	C							
Brown	Dog	C							
Brown	Guinea Pig	C							
Brown	Rabbit	C							
Black	Dog	C							
Black	Guinea Pig	C							
Black	Rabbit	C							
Mohair	Goat	M	17.0	3.00	8.4	0.8	2.9	2.3	
Agouti strain	Rabbit	C							
Human hair		CM	16.4		8.7	1.2	1.9	2.1	1.
Human hair		M			8.9	1.2	3.1		
Human hair		PX			9.8	2.0	4.2	3.3	0.
Human hair, an average		C	16.0	5.25	9.1	0.8	2.7	3.0	1.
Hoof									
Hoof	Horse	C	16.3	2.30					
Hoof	Steer	C	15.9	2.00					
Hoof	Swine	M			9.2	1.9	4.5	5.5	0.
Horn									
Horn	Cattle	C	15.8	2.96	9.6	0.9	3.2	4.6	1
Horn	Rhinoceros	C	15.6	2.40	8.5	0.7	2.8	8.8	1.
Quills									
Quills	Echidna	CM	15.20	4.00	7.4	0.6	1.9	9.6	2.
Quills	Porcupine	C	15.96						
Quills, an average	Porcupine	CM	15.90	3.00	8.1	0.6	2.6	3.3	0.
Quills	Turkey	C	16.03						
Nail									
Nail, Finger	Human	PX			9.2	1.6	3.8	3.8	1.
Nail, an average		C	15.90		8.6	0.5	2.8		
Neurokeratin		CX			4.2	2.9	6.3	7.2	1.
Scales	Tarpon	C	16.30		9.4		4.6		
Shell	Turtle	C	15.10	2.00	6.4	1.7	2.0	14.9	2.
Skin									
Normal; Whole									
Normal	Human	M			5.3	1.2	5.7	1.8	0.
Normal	Human	CPX			3.3	1.8	6.9	1.6	1.
Normal	Human	M			8.1	1.2	4.1	3.2	1.1
Normal, Epidermis	Human	PX			7.9	1.9	7.1	5.0	0.
Normal, an average value	Human	C	14.50	1.24	7.8	0.7	4.3	5.0	1.
Normal, range: Chicken, Duck, Turkey	Fowl	M			5.3– 7.0	0.6 1.3	2.4- 5.0		
Normal	Lamb	C	(16.00)	0.79	15.0	0.7	3.5		
Normal, range: Cat, Dog, Rabbit, Rat, Mouse	Mammal	M			7.6– 8.8	1.1 1.4	3.7 5.2		
Normal	Rat, young	C							
Normal	Rat, adult	C							
Normal, an average value	Snake	CM	16.10	2.30	6.2	0.8	2.5	5.5	1.0
Normal; Layers									
Corium	Human	CPX			2.7	1.3	7.3	1.4	1.0
Epidermis	Human	CPX			3.6	2.4	5.8	3.4	2.
Stratum Corneum	Human	CPX			4.3	2.9	7.9	3.7	2.
Pathological; Whole									
Epithelioma	Human	M			8.4	1.7	4.3	3.8	1.
Melanosarcoma	Human	CPX	(16.00)		2.5	1.7	6.2	5.5	
Psoriasis	Human	C	15.30		12.2	0.8	4.0		

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

PHE	*CYS*	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	10.2													*Cf.* B & B, '51
3.7	11.0	0.3	12.	5.3	7.5	4.6	7.7	8.8	7.1	7.1	3.9	9.5		Schroeder, '55b
6.8	7.6	0.7		7.5	8.5	6.2	7.3			8.9	5.3			Block unpub.
5.5	7.4	0.5	10.2	4.7	8.5	6.4	8.9	10.1	7.6					*Cf.* B & B, '51
4.6	6.7	0.4	14.0	4.7	7.4	5.2	8.8	8.9	5.7	7.1	5.3	9.9		Ward, '55
							9.7	6.6						*Cf.* B & B, '51
4.8	8.4	0.4	12.6	4.6	7.1	4.9	8.4	8.8	6.4	7.1	3.9	10.2		Schroeder, '55b
4.6	7.4	0.3	21.0	4.8	7.3	5.4	8.8	8.2	5.1	7.7		13.0		Ward, '55
4.5	5.4	0.7	16.0	4.1	8.9	3.9	7.8	9.3	6.3	10.1		9.3		Schroeder, '55b
5.3	7.6	0.3	13.9	4.4	8.2	3.6	7.8	8.0	6.5	8.9	6.6	10.1		Schroeder, '55b
5.3	7.6	0.4	11.6	4.1	7.6	3.7	8.1	8.1	6.6	8.4	5.6	10.2		Schroeder, '55b
5.5	8.1	0.4	13.4	4.3	8.9	3.7	8.2	8.4	7.1	9.7	7.3	10.5		Schroeder, '55b
4.8	7.1	0.02	20.0	4.5	8.6	4.2	8.5	7.7	5.7	8.8		12.5		Ward, '55
	7.4							8.7	7.2					Stahl, Priv. Com.
	9.1													Flesch, '50b
	9.0													Flesch, '50b
	11.5													Flesch, '50b
	11.3													Flesch, '50b
	8.8													Flesch, '50b
	11.7													Flesch, '50b
	14.9													Flesch, '50b
	13.9													Flesch, '50b
	9.4													Flesch, '50b
	12.8													Flesch, '50b
	13.1													Flesch, '50b
3.4	3.7	1.3	9.6	4.2	7.7	3.7	5.8	13.4	6.9	4.5	4.0	5.3		Ward, '55
	14.3													Flesch, '50b
2.3	17.6	0.7	10.3	8.3	6.3	4.7	5.3	13.3	3.8	4.0	2.7	4.2	0	Lang, '52
2.3		0.5			6.8	4.2	6.0	15.0		3.6				Dunn, '49d
2.6	16.7	0.7	11.7	8.0	5.8	3.0	5.3	13.8	6.3	4.1	3.5	7.6		Block, unpub.
2.7	16.3	0.8	7.6	7.7				12.7	5.8		4.5	4.3	0	*Cf.* B & B, '51
	5.8													*Cf.* B & B, '51
	6.0			4.9		4.4	5.0							*Cf.* B & B, '51
3.4	3.7	1.3	9.6	4.2	4.8	5.5	6.8	17.8	10.5	8.2	4.7	5.5		Agren, '51
3.1	8.5	1.4	5.6	5.2	8.1	4.6	5.5	14.9	7.9	9.7	2.2			*Cf.* B & B, '51
5.1	8.9	1.5	7.0	5.1	9.3	4.8	5.7	13.5	9.0					*Cf.* B & B, '51
7.2	12.5	1.1	6.0	4.1										*Cf.* B & B, '51
	5.7													Edwards, '44
3.6	9.5	0.8	6.2	4.3				13.5	7.9					*Cf.* B & B, '51
	7.7													Edwards, '44
2.9	10.5	1.0	6.9	6.0	7.6	3.4	4.6	13.2	6.2	3.8	3.7	3.3		Block, unpub.
	11.9	0.6						15.7	8.4					*Cf.* B & B, '51
12.2	4.3	1.6	3.3	9.2	15.3	11.3	5.7	11.9	3.7	5.0	9.1	3.4	0	Block, '51c
														Cf. B & B, '51
5.9	7.7													*Cf.* B & B, '51
2.1	1.3	1.5	3.6	2.7	3.1	2.8	4.2	11.9	4.1	6.2	8.0	3.2		Bocobo, '52
7.0	2.5	1.8	8.6	5.6	8.8	2.3	6.3	13.3	5.2	8.1	7.1	7.9	6.3	Müting, '55
2.5	0.7	1.0		3.6	4.7	2.3	3.8	11.9	6.8	13.7				Liefländer, '54
2.9	3.5	1.0	17.0	3.5	7.9	3.8	5.4	15.9	8.4	11.4	3.0	3.3		Block, unpub.
	3.3	2.6												*Cf.* B & B, '51
3.8–4.6		0.8–1.1		2.8–5.1	6.6–7.9	3.4–6.2	5.8–7.9	10.0-11.0	6.3–9.3	8.0–10.0				Dunn, '49c
		0.9												*Cf.* B & B, '51
2.3–3.0		0.9–1.2		3.0–4.2	4.2–5.6	2.2–2.6	3.3–4.2	11.0-14.0	6.5–7.5	12.0–15.0				Dunn, '49c
	1.2	1.6												Bonting, '50
	0.5	1.2												Bonting, '50
4.1	6.2	1.3			8.3	6.8	5.6	9.1	6.4	13.8				*Cf.* B & B, '51
6.6	2.0	1.8	8.8	4.2	9.3	3.1	6.8	12.8	4.9	9.9	6.6	8.7	7.1	Müting, '55
5.0	4.8	1.9	9.7	5.3	9.0	3.7	6.6	14.1	5.5	7.8	6.5	4.9	4.5	Müting, '55
7.1	9.1	2.0	8.3	6.0	10.2	2.1	6.7	13.2	4.7	3.7	6.9	6.6		Müting, '55
2.6	1.1	0.9		3.9	5.3	2.6	4.2	12.7	7.2	14.0				Leifländer, '54
10.0	0.8	1.0	2.2	6.2	10.0	1.0	4.1	14.2	7.0	2.3	7.5	1.7	0	Müting, '51-'52
	1.9													*Cf.* B & B, '51

	Source	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	PH
KERATINS										
Tooth-Enamel										
Normal		CM	13.6		7.9	1.4	4.4	1.2	0.13	7.
Normal		CM			7.9	1.4	4.4	1.2	0.12	7.
Normal		X				1.2	3.2			
Normal		C			8.1	0.8	2.6			
Normal		M			7.9	1.4	4.4			
Normal		X	(16.0)		12.8	0.9	3.6	2.8		8.
Carious		CM			8.2	1.6	5.2	0.4	0.8	6.
Wool										
Whole										
Whole, range			(16.0)		6.0–10.4	0.6–0.7	2.2–3.3	2.9–6.1	0.7–1.8	1. 4.
Whole, an average value		C	16.3	3.59	9.7	0.8	2.8	5.1	1.5	3.
Whole		M				1.0		4.3	0.6	2.
Whole		M	14.0		10.1	1.2	3.2	5.6	0.7	4.
Whole		X								
Whole			16.9	3.53	10.0			5.4		3.
Whole, Australian-Asian Merino, California-grown		M	17.08		8.6	0.9	2.9	4.0		3.
Whole, Australian Merino 64		CX	16.35		9.5	1.1	3.3	5.4	1.0	4.
Whole, Australian Merino-64, virgin		X	16.62	3.68	10.1	0.9	2.7	6.2	2.0	3.
Whole		M	16.8	3.52	8.7	0.9	2.9	3.8		
Fractions:										
Alkali-soluble fraction			13.4	5.08				4.2		3.
Cementory Material		M	13.4	1.59	7.9		2.7	6.5		5.
Cuticle		X	(16.0)	1.14	7.9		3.6	3.2		2.
Epicuticle			(16.0)		3.2		1.4			0.
Epicuticle		C	13.4	2.4						
Epicuticle, Cortical membrane			(16.0)		11.2					1.
Epicuticle, Para Cortex			(16.0)		15.6					1.
α-Keratose			(16.0)		10.8					1.
Keratin Derivative from Merino-64		X	15.26	2.6	10.2	0.81	3.9	4.7	0.83	4.
Ortho-Cortex			(16.0)		6.6		2.3			1.
Scales			15.2	5.9	5.1			3.5		
Spindle Cells			14.5	8.30	7.7			2.6		1.
Spindle Cells		X	16.1	3.61	10.1		3.6	4.2		2.
Water-extracted fraction, nondialysable		M	15.5	1.0	8.2	0.9		3.2		2.
SHELL PROTEINS										
Crustacea										
Callinectes sapidus, arthropodin		M			4.4	3.8	3.8	5.4		7.
Callinectes sapidus, sclerotin		M			4.5	1.8	2.9	4.3		4.3
Homarus vulgaris (lobster) arthropodin		M			6.6	2.5	2.9	6.2		7.1
Homarus vulgaris (lobster) sclerotin		M			3.6	2.4	2.8	4.3		4.7
Echinoderm										
Arbacia aequituberculata, calcite		M	uncorr.		5.8	1.1	4.8	2.0	1.3	
Mollusk										
Gryphaea angulata, calcite		C	uncorr.		0.5	0	3.6	3.3	0	
Meleagrina margaritifera, aragonite		C	uncorr.		6.5	0	2.5	2.9	2.5	
Meleagrina margaritifera, calcite		C	uncorr.		4.8	1.6	3.1	15.3	3.0	
Ostrea edulis, calcite		C	uncorr.		2.9	0.7	4.3	3.1	0.5	
Pecten maximus, calcite		C	uncorr.		1.6	0.8	10.2	0.4	0	
Pinna nigra, aragonite		C	uncorr.		5.1	0.4	6.8	4.0	1.4	
Pinna nigra, calcite		C	uncorr.		3.0	0.9	4.8	17.0	1.6	
Pinna nobilis, aragonite		C	uncorr.		5.4	1.1	5.0	6.0	1.8	
Pinna nobilis, calcite		C	uncorr.		4.4	1.0	4.3	11.6	1.5	
Turbo marmorata, aragonite		C	uncorr.		7.7	0	4.9	2.5	1.3	
Pinar Murrhuana (Clam), placs		PX	14.40		4.3	1.8	3.1	14.7		2.9
Pinar-Murrhuana (Clam), fibrous		PX	14.40		7.9	2.4	6.8	7.1	0.6	4.5

STRUCTURE PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
0.7	1.2	7.6	5.2	5.3	2.1	3.8	11.0	5.9	22.0	9.6	5.2	5.9	Hess, '53
0.7	1.2	7.7	5.2	5.3	2.1	3.8	11.2	5.9	20.7	9.6	6.4	7.2	Hess, '54
													Losee, '50
													Losee, '50
													Losee, '50
	1.5	8.2	9.1	7.8	2.6	4.0	3.6	3.1	5.2	6.9	8.3	4.1	Stack, '54
0.5	2.7	6.2	4.0	2.8	1.9	3.1	9.9	5.3	22.1	6.7	7.1	9.4	Hess, '54
7.3– 3.1	0.2– 0.7	0.1– 9.5	6.6	9.7– 11.6		2.8– 5.5	12.9– 16.0	2.3– 7.3	0.6– 6.3	4.1– 4.4	4.4– 7.2		Speakman, '49
1.2	0.6	9.4	6.5	8.2	4.4	5.1	12.6	6.7	6.8	4.0	7.5		*Cf.* B & B, '51
2.2	0.5												Monder, '53
	0.5		5.8	8.5	3.6	7.1							Thomas, '51
				7.4	3.0								Simmonds, '53
		16.0		10.1		4.8				3.3	5.0		Lindley, '47
2.2	0.5	7.1	6.1	7.2	3.5	5.5	12.6	6.2	5.1	3.8	6.0		Jones, '51
.0	0.5	10.4	7.0	8.8	3.7	5.6	14.3	6.7	5.4	4.2	6.6		Corfield, '55
.9	0.7	8.7	6.3	7.4	3.0	4.8	14.4	6.4	5.0	3.6	7.0		Simmonds, '54b
.5	0.5	6.8	7.4	7.4	3.6	5.6	12.5	6.1	5.0	3.7	6.1		Ward, '55
1.0				13.7		5.4				4.1	1.3		Lindley, '47
3.0		11.1		6.5	2.9		7.6	5.7	8.2	2.4	4.9		Ward, '55
.0		9.8		7.2	3.1	6.0	13.0	4.8	4.0	4.5	8.5		Ward, '55
3.5		5.8	2.0	0.8		2.1	27.7	24.7	0.7	0.3	2.5		Golden, '54 & Ward, '55
3.5													Schuringa, '52
.1		5.4	2.1	6.2		1.7	11.1	3.9	3.2	1.6	5.6		Golden, '54
7.1		10.5	5.6	8.7		2.4	14.0	5.0	2.7	1.3	5.6		Golden, '54
3.3		10.3	6.6	6.8		1.9	16.5	8.1	6.7	1.1	6.3		Golden, '54 & Ward, '55
		8.0	6.9	8.9	3.4	5.0	19.7	8.2	4.3	4.2	4.8		Simmonds, '55
2.5		10.0	8.2	7.6		4.0	18.6	12.0	9.1	1.9	8.0		Golden, '54
1.4		11.8											Geiger, '44
.8		19		5.4		4.6				3.3	10.0		Lindley, '47
.6		7.3		9.0	4.0		16.5	7.2	3.6	3.6	6.6		Ward, '55
2.2		5.6		10.4	4.1	5.8	18.9	9.5	2.8	5.8	3.1		Ward, '55
	1.0		6.9	9.6	7.2	11.5	19.7	14.3	8.7	8.7	10.8		Duchateau, '54
	0.9		3.4	7.1	6.6	10.7	18.1	13.7	16.3	12.1	12.1		Duchateau, '54
	0.3		6.9	7.2	7.1	8.6	17.3	13.5	10.3	12.0	10.1		Duchateau, '54
	0.5		2.9	6.9	8.2	9.1	21.5	17.0	13.3	13.3	11.6		Duchateau, '54
.9	1.0			3.5		0.8			6.9				Roche, '51b
0	1.8			0.5		1.0			15.7				Roche, '51b
1.1	1.5			3.7		1.3			14.9				Roche, '51b
1.3	1.3			2.4		0.9			20.6				Roche, '51b
1.0	1.6								15.7				Roche, '51b
	1.3	14.0							11.0				Roche, '51b
1.2		7.1							20.8				Roche, '51b
1.5	0.8	6.3							36.0				Roche, '51b
1.1	1.0	7.0							19.0				Roche, '51b
1.3	1.4	6.5							28.3				Roche, '51b
2.0	0.9	9.0							7.9				Roche, '51b
1.5	9.6	2.7	2.8	2.5	2.8	3.6	3.0	9.6	28.0	3.1	3.7	0	Block, Unpub.
36	2.1	6.3	6.1	3.1	2.7	3.5	11.0	21.6	8.2	4.1	10.7	0	Block, Unpub.

	Source	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY
SILK PROTEINS									
Cocoon Fiber		M	(19.0)		1.4	0.60	1.0	10.5	0.40
Fibroin									
from *Anaphe infracta*			18.4		2.0				2.50
from *Anaphe moloneyi*			20.4		0.9				0.20
from *Anaphe venata*			20.2		0.9				0.50
from *Antherea paphia* syn. *mylitta* (Tussah moth)			18.6		6.6				3.60
from *Antherea paphia* syn. *mylitta* (Tussah moth)			(16.0)					8.7–9.7	
from *Antherea pernyi* (Chinese tussah moth)			18.88		4.6	1.30	0.14	9.0	2.30
from *Bombyx mori* (Mulberry silkworm)			18.35		0.8				0.60
from *Bombyx mori* (Mulberry silkworm)		C	17.93			0.20	0.3	10.9	0.50
from *Bombyx mori* (Mulberry silkworm)		M	18.7						
from *Bombyx mori* (Mulberry silkworm)		M	18.6			0.30	0.6		
from *Bombyx mori* (Mulberry silkworm)		M	(19.0)						
from *Bombyx mori* (Mulberry silkworm)		M	(19.0)		0.7	0.30	0.4	11.6	0.20
from *Bombyx mori* (Mulberry silkworm)		PX	(19.0)		0.8			9.0	
from *Bombyx mori* (Mulberry silkworm)		X	(19.0)		0.8	0.30	0.5		
from *Bombyx mori* (Mulberry silkworm)		X	18.28		0.9	0.30	0.5	9.9	0.40
from *Bombyx mori* (Mulberry silkworm)		X	(19.0)			0.10	0.5		
from *Bombyx mori* (Mulberry silkworm), an average		C	18.7		0.9	0.40	0.5	9.2	0.20
from *Caligula japonica*			17.7		4.6				1.10
from *Nephila madagesis*			17.5		4.2				
from Oak silkworm			18.7			1.20	6.4	8.1	1.50
Protofibroin		CPX	M.R.					1	
Sericin									
Sericin		M	(16.5)		4.0	1.30	5.7	3.7	
Fraction soluble in water at pH 4		C	(16.0)		5.5	1.10	1.2	5.3	1.00
Fraction insoluble in water at pH 4		C	(16.0)		5.1	1.30	1.1	4.9	1.10

COMMENTS—STRUCTURE PROTEINS

Collagens: These proteins are unique in their high content of hydroxyproline and appreciable amounts of δ-hydroxylysine. Inskip (1951) claims that wool also contains small quantity of hydroxylysine. Shark-skin fibers differ from animal collagens in that th contain higher quantities of tyrosine and do not readily gelatinize in hot water (Tsuchiy 1953). Tomlin (1953) claims that reticulin is morphologically identical with collagen.

Wool and Hair: The content of some amino acids differs with different wools. Not all the sulfur is accounted for by cystine and methionine sulfur. It appears to be elementa sulfur or a polysulfide group in place of the usual cystine dithio linkage (Cassie, 1955 Gillespie (1953) has reported the preparation of an electrophoretically homogeneous kerat

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	0.1	13.5	2.5	0.8	1.2	2.7	1.8	4.6	28.3	21.0	0.4		Fukuda, '55
		2.5	0.8						24.3	54.0			Lucas, '55
		0.9	0.8						35.7	52.9			Lucas, '55
		0.8	0.5						34.4	52.4			Lucas, '55
		11.8	1.2						20.2	37.5			Lucas, '55
		15.1–15.6											Roche, '50
		12.6	0.17	0.52	0.56	0.74	1.2	6.4	20.3	40.0	0.4		
		14.9	1.6						37.8	30.2			Lucas, '55
		10.9	1.7				0.7		31.7	22.7			Tsintsevich, '50
									37.3				McCoy, '53a
				0.7	1.1	2.9	1.7						Dunn, '49d
									36.7				Shankman, '47
	0.1	12.3	0.9	0.8	1.1	2.4	1.2	1.3	36.0	23.8	0.4		Fukuda, '55
		9.3	2.9	1.6		1.1	2.2	3.2	34.5	29.0			Polson, '51
													Corfield, '54
.13		14.0	1.3	0.7	0.8	2.7	1.5	2.1	37.3	30.2	0.5		Schrœder, '55a
		10.2	0.8	1.7		12.2	23.0	1.8	25.8		0.3		Glanzmann, '52
0	0.1	11.9	1.1	1.2	1.0	2.9	1.9	2.4	34.6	20.8			*Cf.* B & B, '51
		14.7	1.5						17.4	35.3			Lucas, '55
		4.4	1.2						30.3	28.3			Lucas, '55
							4.8		16.3	30.1			Tsintsevich, '50
		7							14	21			Waldschmidt-Leitz, '55
		29.2	5.0	0.9	0.8	1.8	10.3	16.3	8.3	3.9			Fukuda, '55
		13.5		1.7		1.3	2.9	13.8	4.1	4.7			Sasaki, '53
		12.6		1.4		0.9	2.4	13.0	3.8	12.0			Sasaki, '53

om wool. There is no difference in the cystine and methionine contents of the hair from rmal children and those with kwashiorkor (Wysocki, 1954).

Shell Proteins: Clam shells were decalcified with dilute acetic acid and the residue was oroughly washed with water and dried with hot acetone and ether. The remaining protein as mechanically separated into two fractions, that derived from the shell which consisted long fibers and the protein from the hinge which holds the two shells together. These ve been called "fibrous" and "placs." It will be seen that the methionine content of the acs was unusually high (Block, unpublished; *cf.* Beedham, 1954). The fibrous proteins ere digested by both pepsin and by trypsin, although the latter left an appreciable residue. he placs did not appear to be attacked by either pepsin or by trypsin over a period of 3 onths. This resistance to digestion of proteolytic enzymes is reminiscent of the behavior the keratins, yet clam shell placs are low in cystine but extremely high in methionine.

	Source	*Method*	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*P*
ADRENALS										
Adrenals, an average	Human	CPX			2.5	2.6	8.0	2.2	2.4	
Adrenals, an average	Rabbit	CPX			6.1	4.0	6.8	2.8		7
Adrenals	Swine	M			5.6	2.1	6.0			8
BONE										
Raw bone		M			8.1	1.0	4.7			2
Mucopolysaccharide protein complex	Ox-femur	X	11.3	1.83	5.5	3.8	6.0	2.8		4
Marrow, an average	Human	CPX			1.8	3.0	8.4	3.0		6
BRAIN										
Brain	Bovine	M								
Brain	Bovine	M								
Brain	Bovine	M			5.9	2.8	8.3		1.3	5
Brain, an average	Bovine	C	13.9		6.5	2.4	5.9	4.7	1.3	4
Brain, proteolipid proteins	Bovine	PX	9.81		1.5	1.2	4.5	6.8	1.4	6
Brain	Dog	C	13.4		7.0	2.6	6.1	4.7	1.6	4
Brain	Guinea Pig	C	14.8		7.1	3.0	6.4	4.8	1.2	
Brain, an average	Human	C	14.0		6.6	2.6	6.4	4.1	1.4	5
Brain, from Krabbs-Disease	Human	C	12.0		6.6	2.4	3.9			
Brain, from Amaurotic Idiocy	Human	C	14.1		6.1	1.5	5.1	5.1	1.2	6
Brain, proteolipid proteins from	Human	PX	9.63		1.4	1.4	4.6	7.2	1.5	6
Brain	Lamb	M			5.6	2.8	9.3		1.4	5
Brain, an average	Lamb	C	14.2		6.8	2.6	6.2	4.0	1.1	4
Brain, an average	Monkey	C	14.4		6.6	2.5	6.2	4.0	1.2	4
Brain, an average	Rabbit	C	12.8		6.7	2.3	6.7	4.4	1.4	4
Brain, an average	Rat	C	14.5		6.4	2.5	5.9	3.8	1.1	4
Brain	Swine	M			5.1	2.8	7.1			4
Brain	Swine	M			5.2	2.7	9.0		1.3	5
Brain	Swine	M			5.3	2.3	6.2	4.0	1.5	4
Brain, an average	Swine	C	15.4		6.6	2.7	5.5	3.7	1.2	5
CHITIN	Bombay Prawn	PX				6.6	6.9	4.3		5
EYE										
Cornea mucoprotein		M	12.12		4.9	1.4	8.5	0.8	0.8	4
Cornea	Human	M			7.6	0.7	3.8	1.4		2
Lens		C	(16.0)		3.3	1.6	1.6	4.5		
Lens		M			11.3	4.8	5.2	6.8		8
Lens	Bovine	C	(16.0)							
Lens	Bovine	M	(16.0)		10.6	4.0	5.2	6.3	2.2	8
Lens	Human	M	16.6				5.8	5.8	3.3	8
Lens	Human	M			11.1	3.8	5.5	7.4		8
Lens, Albuminoid fraction	Bovine	M	(16.0)		10.8	3.9	5.7	4.7	2.4	9
Lens, α-Crystalline fraction	Bovine	M	(16.0)		10.6	3.7	4.8	5.4	2.5	10
Lens, β Crystalline fraction	Bovine	M	(16.0)		11.2	4.5	5.1	8.3	1.8	6
Lens, Albumin fraction	Bovine	M	(16.0)		11.6	3.2	4.0	6.1	1.6	4
Lens, Cortex		PX	(16.0)		10.0	0 ?	7.4	5.7	0 ?	5
Lens, Nucleus		PX	(16.0)		10.3	0 ?	3.7	6.0	0 ?	5
Mucoprotein	Bovine	PX	13.2		3.3	1.4	2.0	1.7	0.3	3
Sclera mucoprotein		M	12.35		4.9	3.1	9.4		1.2	5
Sclera	Human	M			7.6	0.8	3.8	1.3		2
Vitreous Humor mucoprotein		M	12.35		5.1	2.4	7.2		1.3	4
Vitreous Humor		X	13.4		6.4	3.0	2.9	1.0	1.3	2
GASTRIC JUICE	Human	PX	γ %							
HEART										
Heart	Bovine	CM			6.8	2.4	7.4			
Heart	Bovine	M								
Heart	Bovine	M			6.3	2.6	9.2		1.2	4
Heart	Bovine	M								
Heart, range: Chicken, Duck, Turkey	Fowl	M			5.3– 6.4	2.5– 2.9	7.0– 8.2			4 4
Heart	Human	M			5.6	2.1	8.9	5.1	1.3	4
Heart, an average	Human	CPX			3.3	2.7	7.8	2.5	2.2	7
Heart	Lamb	M			6.5	2.5	8.5		1.2	4
Heart, range: Cat, Dog, Rabbit, Rat, Mouse	Mammal	M			5.2– 6.4	2.5– 3.6	7.6– 8.9			4 4.
Heart, an average	Rabbit	CPX			2.5	2.6	7.2	2.4		7.
Heart	Rat	M			5.9	2.0	7.1			4.
Heart	Swine	M			5.6	2.2	7.5			4.
Heart	Swine	M			5.2	2.7	9.0		1.3	5.
Heart	Swine	M			6.9	1.8	8.9	2.9	0.9	4.
KIDNEY										
Kidney	Bovine	CM	15.6		6.3	2.0	6.0			
Kidney	Bovine	M								
Kidney	Bovine	M								
Kidney	Bovine	M			6.2	2.4	7.9		1.3	4.
Kidney	Bovine	CM	14.2		6.3	2.7	5.5			
Kidney, range: Chicken, Duck, Turkey	Fowl	M			5.6– 6.7	2.7– 3.6	7.6– 8.3			4. 5.

ND ORGAN PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
3.7	2.2	6.3	7.0	10.2	2.2	7.5	14.0	5.7	2.6	7.2	7.6		Müting, '54
3.8	2.3	8.7	7.2	8.6	1.5	7.2	15.5	5.8	1.7	6.2	7.5		Müting, '54
	1.6		3.1	6.7	3.4	4.8	10.0	8.4	8.0				Camien, '49
	0.7	2.5	3.5	2.6	3.0								Williams, H.H.,'55
1.6	1.2	5.1	5.9	10.3	5.2	6.4	16.5	13.6	3.8	5.3	6.0	0	Eastoe, J.E., '54b
2.0	2.1	8.7	5.3	9.6	1.5	6.5	14.1	7.0	1.2	7.2	7.5		Müting, '54
1.3		6.4							4.1	6.4			Alexander, '53
				7.4		4.8							Schweigert, '44
	2.1		5.2	9.1	5.4	5.4							Schweigert, '54
2.2	3.0			7.4	5.1	4.8							*Cf.* B & B, '51
1.0		7.0	4.6	12.8		6.0			7.5	18.0	5.4		Chatagnon, '54
1.4													*Cf.* B & B, '51
1.6													*Cf.* B & B, '51
2.1	3.0		5.8										*Cf.* B & B, '51
													Cf. B & B, '51
													Cf. B & B, '51
1.0		7.7	3.9	13.6		5.4	4.4	25.2	9.1	16.4	5.0		Chatagnon, '53
	2.5		4.8	9.2	5.2	5.5							Schweigert, '54
2.0													*Cf.* B & B, '51
2.1													*Cf.* B & B, '51
2.2													*Cf.* B & B, '51
2.1													*Cf.* B & B, '51
	1.9		3.9	8.4	3.8	5.4	11.7	10.0	4.8				Camien, '49
	2.1		5.2	8.9	5.4	6.0							Schweigert, '54
1.6	2.3		5.6	4.3	4.1	1.6							Edwards, C.H., '55
1.9													*Cf.* B & B, '51
tr.										7.4			Airan, '54
	1.0		2.5	11.9		5.0							Cravioto, '48
	1.1	4.3	2.8	4.7	3.0	3.8	11.0	7.4	18.0				Schaeffer, '50a
													Cf. B & B, '51
	2.4	7.8	4.1	8.0	5.5	5.7	15.8	9.9	5.3				Schaeffer, '50a
				7.0		1.0	15.5			5.0			*Cf.* B & B, '51
1.6	2.2		4.2	7.2	5.6	5.0	12.0	7.8					Schaeffer, '50b
1.5	3.5			7.2	6.0	5.1							*Cf.* B & B, '51
	2.9	7.5	3.8	9.0	6.4	5.3	15.3	9.9	4.8				Schaeffer, '50a
0.7	1.8		6.0	8.3	6.2	6.0	8.0	6.3					Schaeffer, '50b
1.4	1.6		4.4	7.7	5.8	5.3	11.4	7.5					Schaeffer, '50b
1.8	2.5		3.2	6.7	4.7	4.4	13.0	8.6					Schaeffer, '50b
2.1	4.0		5.8	7.0	4.8	3.7	11.5	10.2					Schaeffer, '50b
3.1		5.3	2.6	11.9		5.7	15.5	11.0	4.4	3.2	3.8		Cagianut, '53
3.3	1.7	5.1	3.1	11.3		6.2	16.4	9.7	4.1	3.5	5.2		Cagianut, '53
1.4	1.1	3.0	2.0	5.4	3.5	2.2	8.4	3.3	12.3	2.2	4.7	3.3	Block, unpub.
	1.2		5.9	11.4		6.5							Cravioto, '48
	1.2	3.8	2.7	4.0	2.4	3.7	10.8	6.6	17.5				Schaeffer, '50a
	1.3		5.4	11.8		5.3							Cravioto, '48
		2.7	2.6	6.5	2.2	3.1	1.4	7.4	21.5	2.6	9.9	18.4	Young, '54
		12.3		19.4		10.2	14.0	5.4	10.8				Miller, E., '53
				8.4	5.2	6.3	13.3	6.9					*Cf.* B & B, '51
				8.4		6.3							Schweigert, '44
	2.3		4.9	9.6	5.1	5.6							Schweigert, '54
1.0		4.7							5.0	6.8			Alexander, '53
	2.2– 2.9		3.9– 4.9	8.2– 9.0	4.9– 5.4	5.6– 6.2	13.0– 16.0	10.0– 11.0	5.0– 5.8				Dunn, '49
2.7	2.3	5.0	3.8	7.5	6.0	6.1	16.4	6.0	5.6	10.4	5.0		Bocobo, '52
3.8	2.7	6.8	7.8	11.2	3.5	7.5	14.6	6.3	2.4	7.7	6.4	1.7	Müting, '54
	2.3		4.5	9.1	5.0	5.5							Schweigert, '54
	1.6– 2.5		4.4– 4.8	7.8– 13.0	4.2– 5.2	4.7– 5.9	9.3– 15.0	10.0– 11.0	5.5– 9.4				Dunn, '49c
2.4	1.9	8.1	7.6	9.0	2.2	6.8	15.0	6.0	2.7	6.3	7.0	5.5	Müting, '54
	1.7		4.2	8.6	6.2	6.7							Solomon, '51
	2.3		3.9	8.0	4.4	4.9	13.0	9.5	5.2				Camien, '49
	2.1		5.2	8.9	5.3	6.0							Schweigert, '54
1.0	2.3		4.1	8.0	4.8	5.8							Edwards, C.H., '55
		6.1	4.6									0	*Cf.* B & B, '51
1.4		5.6							6.0	6.3			Alexander, '53
				7.9		5.3							Schweigert, '44
	1.9		4.3	9.1	4.5	5.8							Schweigert, '54
				8.0	5.6	5.3							*Cf.* B & B, '51
	2.0– 2.2		3.9– 5.0	8.3– 9.4	4.6– 5.2	5.9– 6.5	11.0– 12.0	9.5– 11.0	4.5– 6.0				Dunn, '49c

	Source	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY	PH
Kidney, an average	Human	CPX			2.4	4.0	7.4	1.9	1.7	7.3
Kidney	Human	M			5.9	0.9	5	2.9	0.9	3.5
Kidney	Lamb	M			6.0	2.6	8.4		1.0	4.4
Kidney, range: Cat, Dog, Rabbit, Rat, Mouse	Mammal	M			5.2–6.3	2.3–4.2	6.7–7.7			3.5–4.8
Kidney, an average	Rabbit	CPX			2.9	2.9	6.7	2.5		7.0
Kidney	Rat	M			6.9	2.0	7.2			4.9
Kidney, an average	Rat	CPX			2.7	3.6	9.2	2.9	1.5	7.5
Kidney	Swine	M			5.8	2.8	7.9		1.3	4.6
Kidney	Swine	M			5.8	2.2	6.8			4.4
Kidney	Swine	M			6.8	2.2	7.0	2.6	1.4	4.6

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
2.8	2.0	7.2	6.8	9.2	1.6	7.4	13.8	6.8	2.5	7.3	7.7		Müting, '54
1.6	0.7	5.6	2.7	4.2	1.6	2		15.0	3.8	2.2	3.8		Mondolfo, '51a
	2.0		4.7	9.0	5.7	6.0							Schweigert, '54
	2.0–2.5		4.0–4.8	7.6–8.8	4.0–4.8	5.4–6.4	11.0–12.0	9.7–11	5.4–7.3				Dunn, '49c
2.9	2.2	7.8	7.4	8.5	1.8	6.9	14.6	5.8	2.6	7.0	7.4	1.6	Müting, '54
	2.3		4.4	9.2	6.2	7.5							Solomon, '51
2.3	2.1	7.1	6.2	10.0	3.5	7.5	14.7	5.3	2.6	7.1	7.1	2.5	Müting, '54
	2.3		4.4	8.9	5.2	5.7							Schweigert, '54
	2.1		3.5	8.2	4.5	5.4	12.2	9.9	6.4				Camien, '49
1.0	2.3		4.3	8.3	4.4	6.6							Edwards, C. H., '56

	Source	*Meth-od*	*Nitro-gen*	*Sul-fur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*
LIVER										
Mammalian Normal										
Mammalian	Bovine	M								
Mammalian	Bovine	M			5.8	2.8	9.2		1.6	5.1
Mammalian	Bovine	M								
Mammalian	Bovine	M			5.9	2.7	6.0			4.9
Mammalian	Bovine calf	M	2.05		5.9	2.6	7.9		1.4	5.2
Mammalian, an average	Bovine	C			6.5	2.1	6.1	4.1	1.5	5.8
Mammalian	Cat	C	13.9	0.93	6.7	2.2	5.9	3.7	2.9	4.6
Mammalian, Albumin	Cat	C	15.4	1.20	5.9	3.1	10.2	4.4	3.0	4.4
Mammalian, Globulin	Cat	C	14.8	0.82	6.4	2.3	9.3	3.5	2.9	4.7
Mammalian	Dog	C	(14.0)		6.7	1.4	4.0			
Mammalian	Human	M			5.5	2.1	7.9	5.4	1.6	4.8
Mammalian	Human	M			5.3	1.1	5.7	2.4	0.5	4.3
Mammalian, an average	Human	CPX			2.4	4.6	7.7	3.1	2.2	7.5
Mammalian	Lamb	M			6.8	2.9	8.8		1.6	5.8
Mammalian, Various, range: Cat, Dog Rabbit, Rat, Mouse	Mammal	M			5.4–6.5	2.5–2.9	6.9–8.2			4.8–5.5
Mammalian, Meal	Mammal	M			6.0	2.2	7.0	2.6	0.9	4.8
Mammalian, Meal	Mammal	CM	13.0		4.9	2.2	6.0		0.8	4.4
Mammalian, an average	Rabbit	CPX			2.2	3.2	7.4	2.4		7.5
Mammalian	Rat	M	(16.0)		6.4	3.2	8.4	4.0	1.5	5.0
Mammalian	Rat	M			5.3	1.9	7.0			4.8
Mammalian, an average	Rat	C	12.8	1.10			7.8	4.6	1.7	
Mammalian, an average	Rat	CPX			1.2	4.7	9.1	2.6	2.1	7.8
Mammalian, nuclei	Rat	M	(16.0)		7.2	2.7	9.6	3.7	1.2	3.9
Mammalian, large granules	Rat	M	(16.0)		5.6	2.7	8.4	3.9	1.5	4.8
Mammalian, small granules	Rat	M	(16.0)		6.2	3.0	8.2	4.1	1.9	4.7
Mammalian, supernatant	Rat	M	(16.0)		5.6	2.9	8.1	3.7	1.1	4.4
Mammalian	Swine	M			5.8	2.9	8.2		1.4	5.0
Mammalian	Swine	M			5.4	2.5	6.3			4.4
Mammalian	Swine	M			5.4	2.4	6.0			4.7
Mammalian	Swine	M			6.7	1.9	6.8	3.3	1.1	4.6
Amyloid Protein		PX	(16.0)		6.0		4.1	6.2		6.4
Meal residue		C	11.2					4.1	1.6	
Ferritin			8.4 uncorr.		17.7	0.5	4.8	12.2	1.8	1.8
Apoferritin		CM	16.2	0.89	9.1	4.8	7.8	5.0	1.2	6.1
Mammalian Pathological										
Carcinoma, a composite value	Human		14.90		7.1	3.1	6.7	3.4		7.3
Melanosarcoma nodes	Human		(16.00)		2.0	1.0	8.5	8.0	4.5	6.0
Fowl										
Chicken	Chicken				8.9	1.9	8.1	3.3	1.4	4.5
Range, various: Chicken, Duck, Turkey					5.1–6.7	2.7–3.0	7.3–8.0			4.8–5.1
Fish										
Fish, an average	Cod						5.0	3.7	1.4	5.8
Fish	Red cod				7.2	2.7	8.0	1.5	1.2	5.8
Fish	Sturgeon				3.4	1.1	3.4			
Reptile	Iguana	M	10.1		5.0	2.2	7.0		0.7	3.5
LUNG										
Lung	Bovine	M			6.1	2.2	7.8		1.0	4.3
Lung, an average	Bovine	C	15.3		6.4	2.2	5.6			
Lung	Human	M			3.2	0.9	5.5	2.9	0.8	3.1
Lung, an average	Human	CPX			4.3	3.3	8.0	2.7		7.2
Lung	Lamb	M			6.2	2.5	7.4		0.9	5.0
Lung, an average	Rabbit	CPX			3.8	3.2	7.0	3.5		7.5
Lung	Swine	M			5.0	2.9	6.6			4.2
Lung	Swine	M			5.7	2.4	7.5		0.9	4.3
OVARY										
Ovary	Swine	C	15.8		6.4	1.6	5.0			
Ovary	Swine	M			6.0	2.3	6.9			3.9
Ovary	Herring	M	13.6		4.8	1.2	1.8			
PANCREAS										
Pancreas	Bovine	M			5.3	2.2	7.6		1.5	4.6
Pancreas	Bovine	M			5.5	1.8	6.2			3.8
Pancreas, an average	Bovine	CM			6.5	2.1	5.9			
Pancreas, an average	Human	CPX			5.0	3.7	8.1	2.6	2.4	7.5
Pancreas	Lamb	M			5.1	2.3	8.2		1.3	4.3
Pancreas, an average	Rat	CPX			3.8	4.2	7.0	2.7		7.0
Pancreas	Swine	M			5.7	2.2	7.3		2.3	5.0
PITUITARY										
Pituitary, an average	Human	CPX			3.3	4.1	7.3	0.7		7.3
Pituitary	Swine	M			6.1	2.3	6.9			4.1
PLACENTA, an average	Human	CPX			1.5–3.3	2.2 5.0	6.5–8.4	1.8–4.7	1.4–3.0	6.1–7.5
SALIVARY GLAND		C			6.5	2.7	5.8			

D ORGAN PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
				8.4		6.2							Schweigert, '44
	2.4		5.3	10.3	5.5	6.6							Schweigert, '54
.0		5.5							5.8	6.4			Alexander, '53
	2.3		5.1	8.5	4.7	6.1	11.6	8.9	6.3				Camien, '49
	2.0		4.5	9.7	5.1	6.6							Anderson, '53
.2	2.8	5.2	4.3	8.4	4.8	6.0			6.8				*Cf*. B & B, '51
.3	1.3												*Cf*. B & B, '51
.8													*Cf*. B & B, '51
.2													*Cf*. B & B, '51
													Cf. B & B, '51
.9	2.2	5.0	3.8	7.2	5.8	6.3	13.2	5.6	5.3	9.3	4.4		Bocobo, '52
.7	1.1	6.0	2.0	4.8	3.6	3.1	10.0	25.0	2.9	2.8	2.9		Mondolfo, '51a
.8	2.9	8.6	8.5	9.5	2.8	7.5	14.2	6.6	2.3	6.9	7.5		Müting, '54
	2.4		5.3	10.0	5.8	6.7							Schweigert, '54
	2.0–		4.3–	8.9–	5.0–	6.1–	14–	10–	5.2				Dunn, '49c
	2.4		5.0	9.9	5.7	6.6	15	12	6.5				
	1.8		3.7	8.0	4.6	6.4							Williams, H.H., '55
	1.5	3.7											*Cf*. B & B, '51
.8	2.4	8.4	7.0	9.6	2.0	7.2	15.1	5.4	2.7	7.0	7.5		Müting, '54
.6	2.9	4.1	4.3	10.7	4.9	5.8	9.9	8.4	4.0		4.0		Schweigert, '49a
	2.3		4.1	9.4	6.4	7.8							Solomon, '51
.5	4.1						12.2		4.7				*Cf*. B & B, '51
3.0	2.3	8.0	6.6	9.5	3.2	6.8	12.3	5.0	2.2	7.8	7.6		Müting, '54
.5	2.6	3.9	4.2	10.1	4.6	5.2	8.9	8.1	5.6		4.4		Schweigert, '49a
.5	3.0	4.1	4.0	10.5	5.5	5.5	9.2	8.2	4.2		3.9		Schweigert, '49a
.6	2.5	4.0	4.1	9.8	4.4	5.0	9.2	7.8	3.4		3.8		Schweigert, '49a
07	2.7	3.8	3.8	10.1	4.7	5.2	10.3	8.0	3.7		4.2		Schweigert, '49a
	2.3		4.7	10.0	5.5	6.7							Schweigert, '54
	2.2		3.3	8.0	4.4	5.4	11.7	9.4	5.4				Camien, '48
	2.1		4.7	8.5	4.5	5.9	11.9	8.5	5.6				Camien, '48
1.1	2.4		4.9	9.6	5.2	6.2							Edwards, C.H., '55
2.8	0.0	7.0	6.1	12.4		6.6	13.0	8.4	3.8	6.0	7.9		Wunderly, '54b
1.4	2.8												*Cf*. B & B, '51
2.1	2.8								2.1				*Cf*. B & B, '51
1.7	1.9		4.3	19.1	1.4	4.3	17.2	6.8	3.4	1.9	1.5		Mazur, '50
1.6			5.8				10.6	6.9					*Cf*. B & B, '51
0.5	1.0	1.4	4.8	12.2	1.8	7.9	13.2	7.3	1.3	7.8	2.1	0	Müting, '51/'52
2.0	2.5		5.9	9.5	4.8	5.9							Edwards, C.H., '55
	2.0–		3.6–	9.2–	5.2–	6.4	14–	10–	5.2–				Dunn, '49c
	2.4		4.7	10.0	6.2	6.7	16	11	7.8				
1.1	3.4		5.4										*Cf*. B & B, '51
	3.0		4.8	8.4	5.5	5.8							Tarr, '55
													Cf. B & B, '51
	1.9		6.6	8.9	8.1	4.9							Suárez, '54
	1.6		4.0	8.1	4.6	5.7							Schweigert, '54
		6.7	3.8				9.3						*Cf*. B & B, '51
2.5	0.5	3.4	2.0	3.7	1.7	3.2	8.0	19.0	3.5	4.1	3.5		Mondolfo, '51a
3.7	2.4	7.4	8.6	11.3	3.2	7.3	14.4	5.5	2.8	7.6	7.1		Müting, '54
	2.0		4.8	9.1	4.8	5.7							Schweigert, '54
3.8	2.0	8.5	7.3	10.0	1.8	7.2	14.7	5.2	2.6	7.0	6.8	3.7	Müting, '54
	1.3		2.9	7.9	2.9	5.7	10.4	9.0	7.3				Camien, '49
	1.9		3.8	7.8	4.3	6.3							Schweigert, '54
			3.7										*Cf*. B & B, '51
	1.5		3.6	7.1	3.5	5.1	10.6	9.1	8.6				Camien, '49
			5.9										*Cf*. B & B, '51
	1.9		5.0	8.5	5.0	5.7							Schweigert, '54
	1.7		3.6	6.8	4.1	4.9	10.3	9.6	6.1				Camien, '48
			5.0	7.3	6.0	5.5	6.4						*Cf*. B & B, '51
3.1	2.1	6.2	5.0	9.3	1.7	8.1	13.7	6.8	4.3	6.7	6.8	0.2	Müting, '54
	1.8		4.1	8.3	4.4	5.1							Schweigert, '54
3.3	2.2	8.4	7.6	9.0	3.2	6.6	15.7	5.5	3.5	7.2	7.4	4.6	Müting, '54
	1.8		4.6	7.9	5.8	5.9							Schweigert, '54
2.7	2.2	7.4	5.6	7.8	2.2	6.7	14.4	6.4	2.2	7.3	6.4		Müting, '54
	1.5		3.0	7.8	3.4	4.5	12.9	9.4	5.5				Camien, '49
3.0–	1.6–	6.0–	5.0–	8.5	1.2–	6.0–	10.2–	5.5	1.5	5.2–	7.2	3.0–	
5.5	3.0	8.0	6.5	10.0	2.4	7.8	13.2	7.0	4.5	7.3		6.0	Müting, '54
			3.5										*Cf*. B & B, '51

	Source	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY	PH
SPLEEN										
Spleen	Bovine	C	15.7		6.3	2.1	6.1			
Spleen	Bovine	M			5.4	2.4	7.9		1.1	4.
Spleen	Human	M			4.2	0.9	5.0	2.4	0.5	2.
Spleen, an average	Human	CPX			2.8	5.5	8.2	2.0	1.9	8.
Spleen	Lamb	M			5.8	2.7	8.7		1.4	4.
Spleen	Swine Rat	M			5.4	2.4	6.7			4.
Spleen	Swine	M			5.5	2.7	7.9		1.2	4.
Spleen	Swine	M			5.3	2.2	6.5			3.
TESTES										
Testes	Bovine	C	15.4		6.6	2.1	6.1			
Testes	Bovine	M	16.6		13.5	0.6	1.5			
THYMUS	Bovine	C	15.4		7.2	2.4	6.1			
THYROID										
Thyroid, an average	Human	CPX			4.7	2.3	7.4	2.0		7.
Thyroid	Swine	M			7.5	1.8	4.3			5.

COMMENTS—TISSUE AND ORGAN PROTEINS

It may be assumed from the values given in this table that the amino acid pattern each organ or tissue is approximately the same, irrespective of the animal from which it obtained. This means firstly, that the amino acid values obtained from the total organ of on animal can be used as representative of the amino acid patterns of the same organ in othe animal species, and secondly, this observation explains why the quantitative requiremen for essential amino acids among the different species of animals follows the same patter (*cf.* Chapter VII).

ND ORGAN PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
			3.8										
	1.9		4.1	9.0	4.5	5.8							Schweigert, '54
1.8	0.7	5.0	2.5	4.5	2.4	3.0	11.5	24.0	3.7	2.7	3.7		Mondolfo, '51t
3.5	2.6	7.1	7.6	10.8	1.8	7.6	13.6	6.1	2.6	7.2	7.3		Müting, '54
	1.9		5.0	9.4	5.2	5.7							Schweigert, '54
	1.2		3.5	7.5	4.3	6.6							Solomon, '51
	2.0		4.2	9.3	5.6	5.6							Schweigert, '54
	1.8		3.2	7.3	3.6	5.1	11.5	8.8	6.4				Camien, '49
			3.5										*Cf.* B & B, '51
			2.7										*Cf.* B & B, '51
			4.1										*Cf.* B & B, '51
4.3	2.3	7.0	5.5	9.0	2.4	7.4	12.7	5.2	3.4	7.8	8.2	5.5	Müting, '54
	1.2		4.0	9.3	2.8	5.3	12.4	8.4	6.2				Camien, '49

In this connection it is interesting that Nasset (1955a, 1955b) has reported that the pattern of 15 amino acids in gastric and intestinal juices remains essentially unaltered when he animals are fed either a protein-free diet or one in which the protein is the lysine-rich gg albumin or the lysine-poor zein. As Albanese (1948) has previously shown that the roteins of the feces of infants did not change their amino acid patterns when the babies vere fed diets differing rather markedly in amino acid pattern (milk proteins *vs.* wheat luten), we may assume that the amino acid pattern of the proteins of the entire gastric-ntestinal tract remain relatively constant irrespective of the dietary protein (*cf.* Chapter VII).

	Source	*Method*	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*
FOWL									
Chicken									
Embryo (0-4 days)									
Embryo (19 days)									
Embryo, an average		C	(15.0)		5.8	1.4	6.6	4.6	2.1
Day-old		M			6.7	2.0	7.7	2.8	0.9
Week-old		M			6.6	1.7	6.8		1.0
Week-old		M			6.7	1.8	6.1		0.9
4-5 Weeks old		M			6.7	1.9	6.4		0.9
4-5 Weeks old		M			6.4	1.8	6.0		0.9
10 Weeks old		M			6.7	1.8	7.4	2.1	0.7
Adult		C	(16.0)		5	4.0	9.0	3.3	
Adult		M			5.8	1.6	6.3		
Adult (Special rations)		M			6.4	1.8	6.0		0.9
Adult (Special rations)		M			6.7	1.9	6.4		0.9
Adult, pathological (chondrodystrophy)		C	(16.0)						
Duck		M			5.9	1.8	6.0		
Guinea Pig		C	12.6		6.7		5.6		
Various: Turkey, Duck, Chicken, range		M			5.3– 5.9	1.7– 2.0	6.4 8.9		
MAMMAL									
Cat		M			6.3	1.7	5.6		
Dog		M			6.2	2.1	6.3		
Human, range		M			6.5– 6.7	2.3– 2.5	7.0 7.2	2.5 2.6	
Mouse		M			5.9	1.8	7.9		
Pig									
Newborn		M			7.6	2.4	9.0	2.7	0.8
Weanling		M			7.0	2.8	9.5	2.9	0.8
33 Kilo		M			7.1	2.7	8.8	2.3	0.7
93 Kilo		M			6.7	2.6	8.0	2.5	0.7
Rabbit		M			6.3	1.8	6.6		
Rat									
Fetus		C	(16.0)						
Newborn		M			5.6	2.5	7.4	2.6	0.8
Weanling		M			5.7	2.1	8.0	3.6	0.8
2 Days old		C	13.3	0.9	6.2	2.8	6.1	3.0	0.8
23 Days old, an average		C	11.4	1.1	7.1	2.3	6.5	3.4	0.9
100 Days old, an average		C	13.4	1.2	7.1	1.8	5.6	3.1	0.7
540 Days old, an average		C	12.2	1.3	7.3	1.7	6.5	3.2	0.8
Adult		M			6.3	1.9	7.6	2.3	0.7
Adult		M			5.9	1.8	6.1		
Adult, an average		C	14.7	1.03	6.1		5.2		
Various Mammal Carcasses: Cat. Dog, Mouse, Rabbit, Rat, range		M			4.8 5.6	1.7 2.0	5.5– 9.0		
MISCELLANEOUS									
Cockroach		PX	15.1*		3.0	0.9	1.9	2.0	0.6
Copopods		C	9.8						
Chrysalis	Silkworm	PX	11.9		5.7		5.8	6.6	
Earthworm		CPX	11.93		5.7	1.8	5.6	3.7	
Protozoa									
Entamoeba histolytica		X	16.0	1.38	6.7	1.8	11.6	0.4	
Entamoeba histolytica		X	16.0	1.38	6.9	2.0	13.8	0.8	
Proteus flagella		M			5.5		5.5	1.7	
Tetrahymena geleii		M	7.80		5.3	2.2	5.9	4.3	0.9
Tetrahymena pyriformis W		M			1.6	0.8	4.6	4.4	0.8
Snail, Giant African *(Achatina fulica)*, normal		M			9.2– 13.3	1.4– 1.6	8.5– 10.0		
Snail, Giant African *(Achatina fulica)*, stained		M			6.6	1.6	5.6		6.0

* Uncorrected.

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
												0.0	Neuman, '50b
												1.2	Neuman, '50b
2.8													*Cf.* B & B, '51
1.7	1.9		4.2	6.9	4.1	6.9							Williams, H.H., '54
	2.1		4.4	7.3	4.2	4.8							Price, '53
	1.9		4.5	6.9	4.1	5.0							Price, '53
	1.9		4.4	7.1	4.5	5.2							Price, '53
	1.9		4.6	6.9	4.1	5.0							Price, '53
1.8	1.7		3.9	6.3	4.2	6.5							Williams, H.H., '54
									9.3				*Cf.* B & B, '51
	1.6		3.7	7.2	4.2	5.4	11.5	8.2	10.2				Dunn, '49c
	1.9		4.6	6.9	4.1	5.0							Russell, '52
	1.9		4.4	7.1	4.5	5.2							Russell, '52
									6.6				*Cf.* B & B, '51
	1.9		4.3	7.5	4.7	5.7	11.2	9.8	8.2				Dunn, '49c
													Cf. B & B, '51
	1.8–2.5		2.7–4.0	6.7–7.8	3.7–4.5	4.1–4.8	11–12	7.6–10.0	7.7–13.0				Dunn, '49c
	1.8		3.9	6.1	3.3	4.5	11.5	8.7	10.0				Dunn, '49c
	2.1		4.2	6.6	3.8	4.6	12.7	10.4	9.8				Dunn, '49c
							12.3–12.8						Widdowson, '51
	2.0		3.7	7.3	3.7	4.5	12.9	8.8	10.4				Dunn, '49c
1.2	1.7		4.1	7.3	3.5	6.5							Williams, H.H., '54
1.0	1.8		3.7	7.2	4.0	6.1							Williams, H.H., '54
0.9	1.8		3.8	7.1	4.0	5.9							Williams, H.H., '54
0.9	1.9		3.5	6.9	3.8	5.5							Williams, H.H., '54
	1.9		4.1	6.6	3.6	4.7	12.7	8.9	8.9				Dunn, '49c
									10.6				*Cf.* B & B, '51
0.8	1.7		4.1	6.7	3.5	5.6							Williams, H.H., '54
1.9	1.7		3.9	6.6	3.5	5.7							Williams, H.H., '54
1.3	2.0–3.0												*Cf.* B & B, '51
1.7	3.0												*Cf.* B & B, '51
1.4	3.0												*Cf.* B & B, '51
2.0	3.0												*Cf.* B & B, '51
1.5	1.7		3.6	6.2	3.5	5.3							Williams, H.H., '54
	2.0		3.7	6.4	3.8	4.5	13.9	8.1	10.0				Dunn, '49c
2.5			4.5	10.8									*Cf.* B & B, '51
	2.0–2.5		3.6–4.0	6.2–7.1	3.4–4.4	4.2–5.0	11–14	7.8–10.0	6.9–10.0				Dunn, '49c
2.2	3.3	2.3	3.0	3.5	2.4	2.7	7.5	6.8	3.0	4.6	3.0	0	Block, unpublished
1.2	3.0												*Cf.* B & B, '51
1.6	2.3	5.1	5.0				9.0	7.8	3.8	5.3			Block, unpublished
1.0	3.0	3.7	5.0	7.8	5.5	6.7	11.9	8.1	4.7	6.6			Block, unpublished
		6.7	6.2	10.8	2.5	2.2	14.6	13.6	5.3	9.1	7.6		Becker, '54
		6.1	5.8	9.8	2.1	2.4	15.2	12.6	4.9	9.1	6.2		Becker, '54
							12.8	18.9					Weisbull, '51
0.9	1.7	3.9	4.9	8.4	6.1	5.1	15.0	11.9	5.1	4.7	3.1		Wu, '52
	2.7		4.9	7.1	5.7	5.0							Pilcher, '54
	1.0–1.3			4.9–5.8	4.5–5.6	4.3–4.9							Mead, '53
	1.2		4.3	7.5	4.6	5.2							Mead, '53

MMENTS—WHOLE ANIMALS

The striking similarity of the amino acid composition of avian and mammalian tissues is eworthy. The smaller quantities of glycine present in lower animals may be a reflection smaller requirements for glycine.

	Method	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*P*
ESTERASES									
Inorganic pyrophosphatase (Bakers yeast)	X	16.2		3.7	2.5	12.3	6.6	4.0	
CARBOHYDRASES									
Amylase (swine pancreas)	X	15.92*		5.8	3.9	4.9	5.3	6.7	1
Lysozyme	C	16.9							
Lysozyme	C	16.7		12.8	1.0	5.3	3.7	7.2	
Lysozyme	CPX	18.6	2.18	12.7	0.9	5.2	3.3	7.1	
Lysozyme	M	18.6		10.9	0.9	4.9	3.1	9.1	
Lysozyme	M	18.1							
Lysozyme	M	18.6*		11.3	0.8	5.2	3.0		
Lysozyme	M	M.R.		11.2	1.0	5.7	3.0		
Lysozyme	X			12.9	1.1	5.3	2.9		
Lysozyme	X	18.6		13.1	1.2	6.0			
Lysozyme	X			12.4	1.9	5.5	5.2		
Lysozyme	X	M.R.		11	1	5.8	3.2		
Lysozyme (from papaya latex)	X	17.2	1.75	7.9	1.4	4.9	7.9	4.8	
Taka-Amylase A	X	(16.0)		2.7	2.0	5.8	9.2	3.9	
Taka-Amylase A	X	15.0		2.9	2.2	6.3	10.2	4.2	
PHOSPHORYLASES									
Phosphorylase	CM	16.5		11.2	3.2	7.0	5.7	1.9	
NUCLEASES									
Ribonuclease	X	17.8	3.40	4.4	3.8	9.4	6.8	0	
Ribonuclease	X	16.5		5.0	4.1	10.8	8.7		
							8.0		
PROTEOLYTIC									
Carboxypeptidase	X	15.4		4.7	3.2	7.1	9.7	3.4	
Chymotrypsin	M	16.06			1.1		2.7	5.7	
Chymotrypsin	PX			3.0	1.2	6.1	2.9		
Chymotrypsin	PX			2.9	1.6	9.1			
Chymotrypsinogen	M	16.4		2.7	2.3	7.5	2.6		
Chymotrypsinogen	MX			2.8	1.2	8.0	3.0	5.6	
Chymotrypsinogen	PX								
Chymotrypsinogen				2.8	1.1	7.6	2.8		
Leucine aminopeptidase	X	(16.0)		6.1	2.4	9.5	3.0		
Lytic enzyme, crystalline (rabbit spleen)	X	M.R.		6	1	6	3	2	
Pepsin, an average	CM	15.4		1.4		1.4	10.8	2.3	
Pepsin	M	14.1							
Pepsin	MX			1.0	0.9	0.9	8.5	2.4	
Pepsin	PX			1.6	1.3	1.3	8.9	2.3	
Pepsin, heat-precipitated	C	15.2		1.2		2.1	8.7	2.2	
Pepsin, heat-precipitated, filtrate	C	15.4		1.8		1.7	12.0	2.3	
Rennin	X	(16.0)		1.3	2.8	2.9	6.6	1.4	
Papain	X	16.1		7.0	0.8	5.0	13.3	4.3	
Papain	X	16.1		6.8	0.8	4.5	11.5	4.0	
Papain, crystalline	X	16.4		7.4	0.9	5.0	12.5	4.3	
Papain, (given as ratio of AA in %)	PX			9.7	1.2	6.5	16.2	5.6	
Trypsin	PX	14.8		1.6	1.7	6.1	4.5	1.0	
Trypsin Inhibitor (lima beans)	CM	16.2*	5.5	4.1	5.4	6.1	1.7	0.2	
Trypsin Inhibitor (pancreatic)	PX	16.5		15.0	0	7.1	9.0	0	
Trypsinogen		(16.0)		1.6	1.4	10.2	6.4	3.7	
Trypsinogen				1.7	2.0	8.4	6.6		
IRON ENZYMES									
Catalase (horse blood)	CX	16.8		8.4	4.0	7.1	5.1		
Catalase (horse liver)	CX	16.8		8.5	3.7	6.6	5.5		
Cytochrome C, an average	C	15.4		2.8	3.4	19.9	5.6	1.2	
Cytochrome C, (horse liver)	X	15.42		3.9	1.2	16.6	3.3	1.3	
Cytochrome C, a fraction	X	12.5		5.5	1.2	6.8	1.0	2.3	
Ferriporphyrin C, peptide from (beef, salmon)	PX	M.R.			1	1			
Ferriporphyrin C, peptide from (chicken)	PX	M.R.			1	1			
Peroxidase (horseradish)	C	13.2		8.4	0.9	4.9			
DEHYDROGENASES									
Glyceraldehyde-3-Phosphate Dehydrogenase (rabbit)	MX	(16.0)		5.2	5.0	9.7	4.6	2.1	
Glyceraldehyde-3-Phosphate Dehydrogenase (yeast)	MX	(16.0)		6.0	3.5	10.7	5.2	2.2	
Lactic Dehydrogenase (rat liver)	M	14.8		5.2	1.8	9.5	2.7	3.6	
YELLOW ENZYME	C	16.3	0.47	8.1	2.8	13.6	7.7	4.8	
MISCELLANEOUS OXIDATIVE ENZYMES									
Lipoxidase	MX	(16.0)		1.7	3.6	7.8	6.2	0.4	

* Values uncorrected.
† Corrected for hydrolytic losses.

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

'S	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.5	3.7	5.6	6.9	10.2	4.7	10.9	13.8	3.8	4.8	7.5	0.0	Hausmann, '52a
3	2.1	4.1	3.9	11.5		7.8	10.5	14.5	6.7	6.9	3.6	0.0	Caldwell, '54a
								10.0					Friedberg, '50
							2.9	10.2					Fromageot, '49ab
9	2.0	6.2	4.6	6.0	5.3	5.3	2.8	10.2	4.8	5.2	1.1		Fromageot, '50a
9	1.8	5.8	4.7	5.9	4.5	4.1	3.7	15.7	4.9	5.0	1.2	0.0	Lewis, '50
									5.4				McCoy, '53a
	2.2	5.9	5.2	5.3	5.2	3.8			5.0	5.2	1.5		De Fontaine, '54
	2.1	9.6	6.7	7.0	6.9	6.1	4.1	19.8	11.1		2.0		Fox, '55b
0	2.0	5.4	3.9	5.6	4.2	5.6	3.7	10.3	4.1	5.2	1.4		Mills, '52
													Monier, '52
6													Slotta, '52
	1.9	8.6	6.7	8.2	5.9	5.3	5.1	20.4	11.8	12.4	1.9		Thompson, A.R., '55
8	2.0	5.3	4.9	5.1	4.9	2.8	5.4	9.6	5.8	5.6	6.7		Smith, E.L., '55c
	3.9	6.4	10.5	8.1	5.3	4.2	6.8	16.2	6.5	6.7	4.1	0 ?	Akabori, '54a
7	2.3	6.9†	11.4†	8.9	5.5	5.0	7.4	17.7	7.0	7.3	4.5		Akabori, '54b
4	2.6	3.4	4.2	10.2	6.3	7.1	13.2	10.2	3.8	4.6	4.6		Velick, '51a
3	3.6	10.2	8.0	1.8	2.4	6.7	11.1	13.5	1.5	6.9	3.5		Hirs, '54c
5	5.0												Weil, '55
7	0.4	8.7	8.1	8.4	6.9	4.9	9.7	10.5	4.0	4.3	3.2		Smith, E., '54a
2	1.1												Weil, '53
7		10.2	7.8	5.5	2.9	8.8	12.2	16.1	5.1	6.0	3.0	0	Block, unpub.
8			10.4	16.9		9.8	9.1	11.8		7.7	5.4		Keil, '54c
	1.1	10.6	10.4	9.7	6.9	10.1	15.3	10.6	3.1	5.7	3.7		Lewis, '50
6	1.2	11.4	11.4	10.4	5.7	10.1	9.0	11.3	5.3		5.9		Brand, '48
							9.2	11.3		7.6			Vianna, '51
		11.9	10.4	9.3	4.8	9.9	6.5	11.0	6.6	7.3	3.8		Green, N.M., '54
8	1.4	7.7	6.5	10.2	6.6	7.8	12.5	11.8	5.6	8.0	4.8	0	Spackman, '55
	2	10	7	10	7	6	11	20	11	13	5		Jollès, '54
5			9.8				15.4	6.8					B & B, '51
									7.3				McCoy, '53a
.2	1.7	12.2	9.6	10.4	10.8	7.1	11.9	16.0	6.4		5.0		Brand, '48
.5	1.9	12.0	10.4	9.7	8.6	7.6	27.6	10.0	7.7	4.4	5.2	0	Block, unpub.
3													*Cf.* B & B, '51
3													*Cf.* B & B, '51
.8	0.9	7.8	5.6	13.4		9.9	11.5	15.3	4.5	2.7	4.6		Schwander, '52
.9	0 ?	4.9	3.3	5.3	5.2	7.1	10.9	9.8	6.4	4.5	4.3		Smith, E., '54b
.8	0 ?	4.2	3.1	5.0	4.9	6.4	10.0	8.6	6.3	3.7	3.8		Bigwood, unpub.
1		4.9	3.6	5.6	5.5	7.3	11.1	9.7	8.1	4.6	4.4		Close, '53b
.6		6.4	4.6	7.7	7.2	9.6	14.4	12.6	10.6	6.0	5.7		Close, '52
.3	0.8	12.4	4.4	6.6	5.5	4.0	5.8	12.3	6.7	4.0	4.3	1.7	Block, unpub.
.5	0 ?	13.5	5.3	4.9	7.0	1.5	8.7	16.0	0.4	1.9	8.5		Fraenkel-Conrat, '52b
.0	1.6	3.4	4.4	3.0	1.0	3.0	8.0	8.0	7.3	13.0	6.0		Keil, '54b & '54d
.6	1.2		5.4	18.2		9.0	10.7	13.6		5.8	5.7		
	0.9	16.7		6.8	6.6	7.2	6.2	13.1	6.5	4.7	3.5		Green, N.M., '54
.6							10.4	15.7					*Cf.* B & B, '51
.8							9.9	15.7					*Cf.* B & B, '51
.7							16.4	5.3					*Cf.* B & B, '51
.1	6.0	3.1	2.2	10.3	2.5	5.2	8.0	29.2	6.5	2.3	3.2	0 ?	De Barbieri, '51a
.9	4.9	9.6	6.1	5.5	2.7	11.1	7.0	31.5	5.8	1.4	2.7	0 ?	De Barbieri, '51b
			1			2	3			1			Tuppy, '55
		1	1			2	3						Tuppy, '55
													Cf. B & B, '51
	2.7	4.4	5.2			9.2	5.7	10.6	5.9	7.1	3.4		Velick, '53
	2.8	4.7	6.0			10.3	4.3	9.9	4.9	7.3	3.8		Velick, '53
.4	2.3	6.7	2.9	12.6	8.0	11.6	8.6	11.1	4.7		5.5		Gibson, '53
.5							6.8			8.1	6.4	0	*Cf.* B & B, '51
	1.8	tr.	8.9	11.4	8.1	7.8	10.4	6.2	6.3	tr.	5.1		Holman, '50

OMMENTS—Enzymes

Analyses of trypsinogen and chymotrypsinogen by quantitative paper chromatography ve given results similar to those in this table (Keil, 1954a; 1954b).

	Nitro-gen	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*	*CYS*
Alfalfa meal	3.20	0.9	0.32	1.0		0.24	0.84	0.3
Alfalfa meal	2.88	0.8	0.29	0.9		0.21	0.76	0.3
Barley	1.92	0.5	0.25	0.3		0.13	0.62	0.2
Barley	1.44	0.4	0.19	0.2		0.10	0.46	0.1
Blood meal	13.44	3.3	5.02	6.7		1.10	6.00	1.50
Buttermilk, dry	5.12	1.0	0.83	2.2		0.41	1.31	?
Coconut meal	3.52	2.4	0.35	0.6		0.20	0.86	?
Corn	1.44	0.5	0.19	0.3		0.10	0.45	0.16
Corn gluten meal	6.72	1.3	0.88	0.7		0.21	2.23	0.76
Cottonseed meal	6.88	3.9	1.16	1.8		0.52	2.02	0.86
Distillers grains, dry	4.48	1.0	0.80	0.8		0.20	1.90	?
Distillers solubles, dry	4.48	0.9	0.70	0.7		0.79	1.70	0.10
Fishmeal	11.20	5.3	1.54	6.6		0.98	3.08	1.05
Fishmeal	10.40	4.9	1.43	6.2		0.91	2.86	0.98
Fishmeal	9.60	4.5	1.32	5.7		0.84	2.64	0.90
Fish solubles, condensed	5.60	1.5	1.05	1.6		0.31	0.77	0.21
Linseed meal	5.60	2.7	0.70	1.2		0.56	1.47	0.66
Liver meal	10.40	3.9	1.43	6.0		0.85	2.93	1.00
Meat scrap	8.80	3.9	1.10	3.4		0.44	2.04	0.77
Meat scrap	8.00	3.0	1.00	3.1		0.35	1.85	0.60
Meat scrap	7.20	2.7		2.9		0.27	1.67	0.45
Milo	1.60	0.4	0.19	0.3		0.12	0.47	0.15
Oats	1.92	0.7	0.25	0.4		0.14	0.58	0.22
Oats	1.44	0.5	0.19	0.3		0.10	0.44	0.17
Peanut meal	7.04	4.5	0.97	1.4		0.48	2.11	0.70
Peas, dry	3.84	1.4	0.60	1.4		0.19	1.20	0.24
Potato meal, white	1.60	0.4	0.15	0.6		0.14	0.40	0.07
Rice, rough	1.28	0.6	0.18	0.2		0.10	0.37	0.10
Rice bran	2.08	0.7	0.21	0.4		?	0.38	?
Rye	1.92	0.5	0.26	0.4		6.16	0.58	?
Sesame meal	7.20	4.0	0.68	1.3		0.54	3.74	0.59
Skim milk, dry	5.60	1.1	0.84	2.5		0.45	1.58	0.42
Soybean meal	7.20	3.2	1.08	3.2		0.58	2.25	0.66
Sunflowerseed meal	7.20	3.7	0.77	1.8		0.59	2.39	0.72
Tankage	9.60	3.6	1.56	3.6		0.42	2.70	0.50
Wheat	2.08	0.5	0.26	0.4		0.16	0.55	0.22
Wheat	1.60	0.4	0.20	0.3		0.12	0.42	0.17
Wheat bran	2.56	0.9	0.30	0.6		0.21	0.16	0.19
Wheat middlings	2.56	0.9	0.34	0.7		0.19	0.62	0.19
Whey, dry	1.92	0.3	0.14	0.9		0.11		0.31
Yeast, dry	7.20	2.0	1.26	3.1		0.58	1.84	0.45

* See also this section "CEREALS, GRAINS, GRASSES, FEEDS and FORAGE and other tables.

IMAL FEEDS*†

alculated as per cent of feed)

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

IET	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*
0.32		0.70	1.3	0.90	1.00						
0.29		0.63	1.2	0.81	0.90						
0.14		0.42	0.8	0.53	0.62						
0.10		0.31	0.6	0.40	0.46						
1.00		3.80	13.9	0.95	8.25						
0.67		1.40	3.0	2.40	2.46						
0.33		0.64	1.9	0.79	1.08						
0.18		0.63	1.1	0.35	0.41			0.3			
0.84		1.13	6.6	2.14	2.18						
0.65		1.25	2.4	1.42	2.19			2.3			
0.45		1.10	2.3	1.90	1.80						
0.41		1.00	1.6	1.70	1.60						
1.90		2.94	5.6	4.04	3.85			4.9			
1.75		2.73	5.2	3.75	3.58			4.6			
1.62		2.52	4.8	3.46	3.30			4.2			
0.56		0.77	1.7	0.98	1.02						
0.60		1.09	1.9	1.44	1.75						
1.05		2.27	5.1	2.60	4.03						
0.75		1.82	3.5	1.87	2.75			8.3			
0.65		1.65	3.2	1.70	2.50			7.5			
0.50		1.49	2.8	1.53	2.25			6.7			
0.16		0.31	1.6	0.46	0.53						
0.18		0.41	0.9	0.53	0.68			0.5			
0.13		0.31	0.7	0.40	0.51			0.4			
0.44		1.23	2.8	1.84	1.98			2.4			
0.24		0.94	1.8	1.20	1.20						
0.10		0.47	0.6	0.25	0.36						
0.11		0.31	0.7	0.38	0.51			0.8			
?		0.29	0.6	0.40	0.57						
0.16		0.36	0.7	0.48	0.53						
1.40		1.62	3.4	2.16	2.30			4.2			
1.05		1.40	3.5	2.00	2.20						
0.67		1.76	3.4	2.30	2.21			1.6			
1.53		1.67	2.9	2.30	2.43						
0.66		1.92	5.1	2.04	3.40						
0.17		0.36	0.7	0.47	0.53						
0.13		0.28	0.6	0.36	0.41						
0.17		0.37	0.8	0.51	0.72						
0.16		0.45	1.0	0.64	0.72						
0.15		0.59	1.2	0.67	0.66						
0.90		2.30	3.3	2.58	2.49						

† Values compiled from the literature.

	Meth-od	Nitro-gen	ARG	HIS	LYS	TYR	TRY	PHI
CEREALS								
Barley cereal	M	1.85	4.2	1.7	3.2		1.2	5.3
Barley cereal	M	2.66	4.2	2.0	4.3		1.3	[illegible]
Barley cereal	M	2.44	4.1	1.7	3.1		1.1	5.6
Barley cereal	M	1.89	4.0	1.7	2.8		1.3	[illegible]
Bread, white, 12 pooled samples	PX		3.4	2.7	2.5	3.0	0.9	4.6
Bread, whole wheat, 11 pooled samples	PX		4.5	2.9	2.5	3.1	0.8	4.2
Corn cereal	M	1.15	4.0	2.8	2.8		0.8	4.9
Cornflakes, raw cereal for (General Foods)	M		3.6	3.2	1.9			5.3
Cornflakes (General Foods)	M		2.1		0.9			5.4
Cornflakes (unspecified brand)	M		4.7	2.7	4.4	4.9	1.0	4.0
Cornflakes (Laukoff)	M		3.1	1.7	2.7	4.7	0.7	3.3
Farina (General Mills)	M		2.7	2.5	2.0			5.2
Farina, puffed (General Mills)	M		2.6	2.2	1.9			4.9
"Grape-Nuts," raw cereal for	M		3.7	2.3	2.8			4.7
"Grape-Nuts"	M		3.5	2.3	1.6			4.9
"Mull-Soy," powder (Borden)	PX		8.3	3.7	5.0	5.2	1.4	4.8
"Muffets" (Quaker Oats)	M		3.6	2.5	2.8			4.4
Oat Flour (Quaker Oats)	M		4.5	2.3	3.6			4.9
"Oaties" (Quaker Oats)	M		5.4	2.5	3.3			5.5
Oatmeal, average of five samples	M	2.9	6.4	2.1	4.1		1.4	5.1
"Pablum"	M	2.44	4.6	2.0	3.5		1.2	4.5
"Pastina, Baby Egg"	M	2.06	4.6	1.9	2.6		1.4	5.5
Rice (Quaker Oats)	M		6.0	2.8	3.8			4.7
Rice flakes (Quaker Oats)	M		4.3	2.2	2.5			4.3
Rice cereal	M	1.23	7.9	2.6	5.8		2.1	5.5
Rice cereal	M	1.04	7.4	2.0	4.0		1.2	4.4
Rice cereal	M	0.99	8.9	2.1	3.7		1.5	6.3
Wheat flakes cereal	M		5.3	2.0	4.1	3.4	1.2	3.7
Wheat for flakes (General Mills)	M		3.9	2.4	2.9			4.4
Wheat flakes (General Mills)	M		3.1	2.2	1.2			4.8
Wheat for flakes (Pillsbury)	M		3.9	2.4	2.7			5.0
Wheat flakes (Pillsbury)	M		2.6	2.2	1.5			5.0
Wheat for puffing (Pillsbury)	M		4.1	2.5	2.5			4.9
Wheat, puffed (Pillsbury)	M		2.7	2.4	1.1			4.7
Wheat for shredded wheat (Quaker Oats)	M		3.3	2.6	2.7			4.2
Various Cereals:								
(Beech-Nut)	M	2.81	5.5	2.1	4.0		1.0	4.4
(Clapp's)	M	2.10	4.7	2.3	4.1		1.3	4.9
(Gerber)	M	2.31	4.5	2.0	2.9		1.2	4.7
(Heinz)	M	1.99	5.4	2.2	3.7		1.3	4.8
MIXED VEGETABLES AND MEAT								
"C" Ration No. 1 (U.S.A.), fresh	M		3.6	2.7	5.7			4.0
"C" Ration No. 1 (U.S.A.), fresh	M		4.3	3.0	6.7			4.6
"C" Ration No. 1 (U.S.A.), stored 3 mos. at 100°F	M		3.4	2.7	5.5			4.4
"C" Ration No. 1 (U.S.A.), stored 3 mos. at 100°F	M		3.4	2.7	5.7			4.2
Chicken Soup	M	0.28	4.4	2.2	5.4		1.2	4.8
Chicken Soup	M	0.35	6.0	2.2	5.5		1.3	4.6
Chicken Soup and Vegetables	M	0.31	4.8	1.9	4.3		1.3	4.1
Dehydrated Corned Beef Hash	M		2.4	1.3	3.2			1.7
Dehydrated Meat and Rice	M		2.1	1.1	2.8			1.5
Liver Soup and Beef	M	0.54	5.1	2.6	6.8		1.3	4.5
Liver Soup, Vegetables and Bacon	M	0.43	4.9	2.3	6.0		1.3	4.9
Liver Soup, Vegetables, Rice and Barley	M	0.45	5.3	2.4	7.0		1.2	4.9
Vegetables and Bacon	M	0.22	4.5	1.9	4.4		1.2	3.8
Vegetables and Bacon	M	0.14	6.3	2.0	5.0		1.5	4.8
Vegetables and Beef	M	0.51	4.1	2.7	5.3		1.9	3.4
Vegetables and Beef	M	0.39	5.7	2.2	6.1		1.3	4.5
Vegetables and Beef	M	0.35	6.3	2.4	6.3		1.2	4.4
Vegetables, Beef and Cereal	M	0.36	5.8	3.1	6.2		1.1	3.9
Vegetables, Lamb, Milk and Cereal	M	0.32	5.5	2.3	6.5		2.0	3.9
Vegetables, Lamb, Rice and Barley	M	0.36	5.8	2.2	6.7		1.1	4.2
Vegetables, Lamb, Milk and Rice	M	0.33	6.3	2.3	6.7		1.2	4.1
Vegetable Soup	M	0.18	5.6	1.8	4.0		1.1	4.4
Vegetable Soup and Lamb	M	0.31	5.1	1.9	5.6		1.3	4.3

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

MET	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
1.3		3.3	8.0	4.4	4.6							Anderson, '53
1.5		3.5	7.7	4.3	5.3							Anderson, '53
1.2		3.1	7.6	4.1	4.4							Anderson, '53
1.1		3.0	7.7	4.0	4.6							Anderson, '53
2.2	4.3	3.1	6.3	4.1	3.8		4.4	3.1	3.1			Block, unpub.
2.3	4.5	3.5	7.3	4.2	4.7		5.3	3.6	3.9			Block, unpub.
2.6		3.8	13.3	4.3	5.0							Anderson, '53
2.2		3.9	1.5	5.0	5.3	23		5.3				Dunn, '49d
2.2		3.7	1.6	4.9	5.2	23		5.3				Dunn, '49d
0.8		3.3	13.3	4.0	5.0							Williams, H.H., '55
1.0		2.8	10.5	3.1	3.9							Williams, H.H., '55
1.2		2.8	6.9	4.9	4.4	38		3.4				Dunn, '49d
1.2		2.7	6.9	4.8	4.2	36		3.6				Dunn, '49d
1.4		3.5	6.6	4.8	4.6	30		5.8				Dunn, '49d
1.4		3.2	7.0	4.9	4.9	31		5.0				Dunn, '49d
1.5	5.1	4.8	7.3	4.3	4.3	14.2	11.2	4.1	4.2			Block, unpub.
1.3		3.2	6.8	4.7	4.9	27		5.4				Dunn, '49d
1.4		3.3	7.1	4.8	5.4	20		5.1				Anderson, '53
1.4		3.6	7.3	5.4	5.6	22		5.2				Anderson, '53
1.6		3.8	9.3	5.1	5.9							Anderson, '53
1.2		3.1	8.0	4.2	4.5							Anderson. '53
1.1		3.1	7.4	4.4	4.7							Anderson, '53
1.4		3.7	7.5	5.1	6.1	16		4.9				Dunn, '49d
1.5		3.1	6.9	4.6	5.4	15		4.5				Dunn, '49d
2.9		4.3	10.1	5.6	6.2							Anderson, '53
1.8		3.1	8.1	4.0	5.7							Anderson, '53
2.4		3.9	10.0	5.0	6.8							Anderson, '53
0.9		2.6	8.0	3.1	4.7							Williams, H.H., '55
1.3		3.3	6.8	4.6	4.7	28		5.2				Dunn, '49d
1.3		3.1	7.1	4.8	4.8	32		5.0				Dunn, '49d
1.3		3.4	6.8	5.0	4.5	32		4.0				Dunn, '49d
1.4		3.2	6.8	5.0	4.5	33		4.7				Dunn, '49d
1.4		3.1	6.7	4.9	4.6	31		4.0				Dunn, '49d
1.2		2.6	6.2	4.6	4.1	30		3.8				Dunn, '49d
1.3		3.1	6.4	4.6	4.7	27		4.5				Dunn, '49d
1.4		3.3	7.6	4.1	4.7							Dunn, '49d
1.5		3.4	8.8	4.6	4.9							Dunn, '49d
1.3		3.0	7.5	4.3	4.4							Dunn, '49d
1.9		3.9	10.6	5.5	5.2							Dunn, '49d
1.6			7.6	5.9	5.1	17		4.4				Dunn, '49d
1.5			8.1	6.3	5.5	18		5.4				Dunn, '49d
1.6			7.9	6.1	5.4	17		4.8				Dunn, '49d
1.3			7.6	5.8	5.2	17		5.1				Dunn, '49d
1.4		3.6	8.7	4.8	5.4							Anderson, '53
1.6		3.5	7.8	5.0	5.5							Anderson, '53
1.3		2.9	7.0	4.2	4.4							Anderson, '53
0.78			3.4	2.7	2.3	6.1		2.8				Dunn, '49d
0.78			2.8	2.2	2.0	5		2.2				Dunn, '49d
1.6		4.2	8.3	4.5	5.4							Anderson, '53
1.4		4.1	8.6	4.5	5.6							Anderson, '53
1.3		4.2	8.8	4.6	5.6							Anderson, '53
0.7		3.4	7.1	4.1	4.6							Anderson, '53
1.0		3.5	8.5	5.4	6.0							Anderson, '53
1.3		3.4	6.9	3.7	2.9							Anderson, '53
1.3		3.8	8.1	4.9	5.3							Anderson, '53
1.5		3.7	8.1	5.0	5.4							Anderson, '53
1.1		3.9	7.5	4.3	4.4							Anderson, '53
1.6		4.3	9.0	5.0	3.8							Anderson, '53
0.9		4.2	8.0	5.4	4.9							Anderson, '53
1.2		4.2	8.2	4.8	5.3							Anderson, '53
0.6		3.5	7.3	4.5	5.3							Anderson, '53
1.1		3.7	8.2	4.1	5.0							Anderson, '53

Native Name or Diet	*Scientific Name*	*Meth-od*	*Nitro-gen*	*ARG*	*HIS*	*LYS*	*TYR*
ALGERIA							
Average Diet*			8.16	4.7	2.2	4.0	
CEYLON (see also INDIA)							
Agati Leaf		M	5.70	5.8	1.9	4.0	
"Brinjal"		M	2.62	3.4	1.7	1.6	
Drumstick Bean		M	3.50	3.6	1.1	1.5	
"Murunga" Leaf		M	5.00	6.0	2.1	4.4	
"Kankun" Leaf		M	4.70	4.9	1.6	3.9	
CHILE							
Average Diet*			11.05	5.2	2.3	4.5	
CUBA							
Composition of 116 Cuban foods of vegetable origin							
EGYPT							
Average Diet*			11.83	5.4	2.4	3.8	
GREAT BRITIAN							
R.A.F. Diet,* mean				5.4	1.4	5.7	2.6
INDIA							
Average Diet*			8.97	6.3	2.1	4.4	
Aconite Bean	*Phaseolus aconitifolius*	M	3.91		3.3	4.6	5.6
Amaranth Leaf	*Amaranthus gangeticus*	M	4.76	4.2	2.2	4.4	3.3
Bengal Gram†	*Cicer arietinum*	CM	3.83	5.8	2.9	7.8	
Black Gram†	*Phaseolus mungo*	CM	4.33			5.9	
"Boi" (fish)			15.70	6.6	1.9		1.6
"Cholam"	*Sorghum vulgare*	M	1.21		3.4	1.6	
"Cumbu"	*Pennisetum vulgare*	M	1.81		3.8	1.8	
Green Gram†	*Phaseolus radiatus*	CM	4.21	6.3	2.7	7.3	
"Halwa" (fish)		M	14.18	3.7	1.3		1.3
"Khesari"	*Lathymus sativus*		4.62			5.3	
Lentils†		CM	4.27	7.9	2.2	6.2	
"Masur"	*Lens esculenta*					5.0	
"Mushi" (fish)		M	14.03	5.2	1.7	16.5	1.5
"Pakat" (fish)		M	14.58	5.2	1.7	19.5	1.4
"Pomfret" (fish)		M	15.02	6.1	1.8		1.3
"Ragi"†	*Eleusine coracana*	M	1.28	1.5	1.8	2.4	
"Rawas" (fish)		M	14.67	7.4	1.7	19.8	1.6
INDOCHINA							
Average Diet*			7.69	7.1	2.3	4.9	
JAPAN							
"Hirame" (turbot)	*Paralichthys olivaceous*	M	17.60	8.7	2.6	9.1	1.2
"Katsuo" (bonito)	*Katsuoonus vagans*	M	17.40	9.4	6.2	10.0	1.6
"Ma-aji" (horse mackeral)	*Trachurus japonicus*	M	16.10	9.3	3.0	10.0	1.7
Ryokuto Bean	*Phaseolus mungo* var. *radiatus*	C	14.90	6.2	2.1	3.3	2.4
"Surume-ika" (squid)	*Ommastrephes sloani pacificus*	M	16.30	11.4	2.5	8.9	1.6
"Yari-ika" (squid)	*Loligo bleekeri*	M	14.60	12.6	2.3	10.0	1.5
JAVA							
Average Diet*			7.36	6.5	2.3	4.3	
LIBYA							
Average Diet*			10.23	5.3	2.2	5.0	
MAURITIUS							
Average Diet*			7.53	5.5	2.3	4.3	
MEXICO							
"Acociles" (crustacea)		M	8.58	6.3	1.8	5.0	2.8
"Ahuahutle" (insect eggs)		M	11.43	7.7	3.3	3.5	11.1
"Alcoga"	*Beta vulgaris*	M	4.24	2.5	1.3	2.8	
"Alegria" (seed)	*Amaranthus paniculatus*	M	2.38	5.5	2.2	4.3	
"Axayacatl" (dried insects)		M	10.10	5.5	2.4	4.3	4.5
"Bagre" (fish)	*Ameiurus* sp.	M	12.13	5.6	2.3	10.6	
"Berros"	*Nasturtium aquaticum*	M	5.61	3.1	2.0	5.2	
"Blanco" (fish)	*Chirostoma estor*	M	9.94	4.3	2.7	9.2	
Broad Bean†	*Vicia fabia*	M	4.22	7.7	2.7	5.9	3.1
"Capomo" (seed)	*Brosinum alicastrum*			7.8	1.3	3.7	
"Charales" (small fish)		M	13.55	4.8	2.3	8.0	3.0
"Chile jalapeña"	*Capsicum annum*	M	1.92	2.0	1.2	2.9	
"Chile pablano"	*Capsicum annum* var. *grossum*	M	2.00	2.0	1.1	3.0	
"Chiles"		M	0.18	2.0	1.1	3.1	
"Espinacas"	*Spinacea oleracea*	M	4.63	4.5	1.9	4.1	
"Frijoles" (beans)		M	1.66	4.7	2.4	6.1	
"Gusanos de Maguey" (butterfly eggs)		M	13.90	3.0	1.6	3.6	4.2
"Guaje"	*Leucaena esculenta*	M	4.73	8.5	2.0	4.1	
"Hongo—orejo de puerco	*Lactarius* sp.	M	5.37	0.48	0.62	2.0	
Iguana		M	12.63	5.6	3.2	9.4	
"Jumiles" (dried insects)		M	13.26	2.9	3.0	3.8	4.0

ENOUS FOODS AND DIETS (see Chapters VII and VIII) (Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

PHE	*CYS*	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
4.9		1.6		3.4	7.9	4.6	5.1							Brock, '52
6.0	1.5	1.4		4.8	8.9	6.2	6.8							Baptist, '54b
4.3		0.9		3.7	6.3	5.1	5.9							Baptist, '54b
4.3	2.7	1.4		3.9	6.5	4.4	5.4							Baptist, '54b
6.4	3.3	2.0		4.9	9.3	6.3	7.1							Baptist, '54b
5.8	2.1	1.7		4.9	8.0	5.7	6.3							Baptist, '54b
4.8		1.9		3.6	7.5	4.6	5.1							Brock, '52
														Navia, '55
4.9		1.7		3.6	9.8	4.5	5.3							Brock, '52
4.2	1.2	1.7		4.8	13.4	4.6	4.3							Cuthbertson,
5.0		1.7		3.8	8.5	4.8	5.9							Brock, '52
4.5	4.9	0.7			5.5	4.1	3.2							Deshpande, '54
3.0	0.7	1.2		2.4	6.5	5.1	5.0							Deshpande, '54
4.8	0.8	1.2		2.5	8.0	6.0	5.4							
4.9	0.7	1.2		3.2	8.9	5.0	6.0							
4.2	0.5	4.4		7.1	11.3	5.9	5.5							Master, '54
5.1		1.6			13.0	6.1	5.9							Balasubramanian, '52ab
4.3		1.8			9.5	5.9	6.2							Balasubramanian, '52ab
5.2	0.5	1.1		3.4	7.9	5.3	6.1							
2.1	tr.	1.8		4.1	6.2	3.4	2.9							Master, '54
2.3	0.7	0.2		2.6										Esh, '53 & '54
4.5	0.8	0.7		3.4	6.5	4.8	5.2							
4.0				2.8										Esh, '54
3.7	0.9	2.0		5.4	7.9	5.4	4.3							Master, '54
2.7	1.0	1.6		4.6	12.5	5.2	3.5							Master, '54
3.5	1.3	3.0		6.9	10.7	5.2	5.0							Master, '54
2.9		1.5		0.0	9.5	6.4	6.7							
4.5	2.3	3.7		8.1	8.8	7.3	6.5							Master, '54
5.0		2.2		4.1	8.7	4.9	6.3							Brock, '52
1.7		1.9	2.4	2.0	3.6	2.6	6.3	6.0	5.8	4.3		4.2		Sugimura, '54
2.2		2.1	2.8	2.4	4.6	3.7	7.8	6.4	6.8	5.7		4.7		Sugimura, '54
2.1		2.0	2.5	2.2	4.1	3.2	7.4	6.3	6.5	4.8		5.0		Sugimura, '54
3.1	0.4	0.5			5.4	3.2	4.1	18.9	8.1	5.6	4.3	2.4		Sasaki, '41
2.0		1.4	2.5	2.3	4.7	3.2	6.8	6.9	5.5	5.9		5.0		Sugimura, '54
2.1		1.1	3.2	2.3	5.3	3.6	7.7	8.2	7.1	5.9		4.3		Sugimura, '54
5.0		1.9		4.0	9.6	4.4	5.9							Brock, '52
5.0		1.9		3.8	7.8	5.0	5.5							Brock, '52
4.9		1.8		3.6	7.9	4.7	5.3							Brock, '52
3.2		1.4		3.9	6.2	5.7	4.0							Massieu, '51
3.4		1.5		4.0	8.0	5.0	6.0							Massieu, '51
3.3		0.6		4.1	5.4	4.3	3.9							Suárez, '54
5.9		1.6		5.2	6.0	3.4	3.8							Suárez, '54
3.2		1.6		4.4	8.0	5.9	5.5							Massieu, '51
5.1		3.1		4.7	9.6	7.9	6.5							Suárez, '54
3.1		1.1		4.6	7.7	4.5	5.6							Suárez, '54
5.1		2.9		6.8	7.5	6.4	3.7							Suárez, '54
4.3	1.2	0.4	5.7	3.1	8.9	6.3	5.1	13.7	6.5	3.7	2.6	4.5		Massieu, '49 & '50
4.0		0.5		3.3	9.2	4.8	8.2							Massieu, '50
3.1		2.1		4.4	7.4	5.3	5.2							Massieu, '51
4.2		0.6		4.5	4.2	3.6	2.8							Suárez, '54
4.7		0.3		4.0	3.6	3.7	2.7							Suárez, '54
4.8		0.4		4.1	3.7	4.1	2.8							Cravioto, '51
6.0		1.4		4.9	9.4	5.0	4.8							Suárez, '54
5.3		0.7		4.8	11.8		5.1							Cravioto, '53
3.7		1.0		3.3	5.2	4.9	4.7							Massieu, '51
3.0		0.2		2.9	6.3	5.8	3.0							Suárez, '54
0.4		0.5		3.6	3.2	4.7	6.6							Suárez, '54
3.3		2.6		7.5	9.7	9.2	5.3							Suárez, '54
2.5		1.5		2.8	6.2	4.5	4.8							Massieu, '51

Native Name or Diet	Scientific Name	Method	Nitrogen	ARG	HIS	LYS
MEXICO						
"Malva"		M	0.26	5.1	[illegible]	4.2
Manioc (bitter cassava)	*Manihot esculenta*	X	0.32	3.7	1.2	3.5
"Nopales"	*Opuntia* sp.	M	1.96	2.9	1.5	4.6
"Parota"	*Enterolobium cyclocarpum*	M	6.44	7.1	2.5	4.8
"Pataste" (seed)	*Theobroma bicolor*	M	7.26	9.3	1.5	4.9
"Pulque"		M	0.99	2.9	1.3	4.4
"Romeros"	*Dondia* sp.	M	3.79	2.4	1.4	3.4
"Tortilla"†		M	3.83	3.5	1.9	2.6
MOROCCO						
Average Diet*			11.20	4.9	2.3	4.6
NIGERIA, NORTHERN						
Average Diet*			11.83	4.9	2.3	3.1
Average Diet*			14.71	4.7	2.2	2.7
RHODESIA, SOUTHERN						
Average Diet*			10.83	4.7	2.5	4.1
TANGANYIKA						
Average Diet*			9.94	6.1	2.4	5.1
UNITED STATES						
Self-selected Diet*		M	10.40	5.4	3.2	8.6
Self-selected Diet,* Older women		M				7.1
Self-selected Diet,* Female, mean		M	11.24			5.8
Self-selected Diet,* Female, maximum		M	15.20			8.6
Self-selected Diet,* Female, minimum		M	8.18			2.6
Mixed Diet, College women		M	(16.00)	5.2	2.8	5.9

* The nitrogen values in those diets which have been starred (*) indicates the quant
of nitrogen consumed per day and not the amount in the mixed diet.

NOUS FOODS AND DIETS* (see Chapters VII and VIII) (Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

HE	*CYS*	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
4.5		0.8		4.2	7.0		4.9							Cravioto, '53
2.2	0.6	1.0	1.9	2.1	2.9	2.0	2.6	12.8	4.5	2.4	4.7	1.5		Close, '53a
5.4		0.7		4.8	5.2	4.0	3.8							Suárez, '54
3.3		0.9		2.9	11.4	5.5	3.9							Massieu, '50
5.8		0.6		3.3	8.7	4.6	5.3							Massieu, '50
3.0		1.2		1.6	3.2		1.7							Cravioto, '53
4.5		0.5		3.4	5.9	4.4	3.5							Suárez, '54
4.4		1.9		4.1	16.2	6.0	5.3							
5.0		1.8		3.6	8.8	4.9	5.0							Brock, '52
4.1		1.8		3.8	9.6	4.3	5.3							Brock, '52
4.1		1.7		3.8	9.9	4.3	5.2							Brock, '52
4.7		2.0		3.9	11.8	4.5	5.5							Brock, '52
4.8		1.8		3.9	7.8	4.7	5.4							Brock, '52
4.0		2.6		4.0	8.0	6.2	6.2							Futrell, '52
4.3		3.0		3.7	7.3	4.3	5.1							Mertz, '52a
3.1		1.5		2.5	5.4	3.7	4.3							Reynolds, '53
4.9		2.5		3.4	7.4	7.3	5.9							Reynolds, '53
2.2		0.8		1.3	4.3	1.6	3.1							Reynolds, '53
4.1		1.5		4.3	7.2	4.6	5.0							Wharton, '53

average value compiled from tables in this book.

	Meth-od	*Nitro-gen*	*Sul-fur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*	*CYS*	*MET*	*SER*
Actinomycin:												
"AC"	M	M.R.										
"AA"	M	M.R.										
"C_1"	PX	M.R.										
"C_2"	PX	M.R.										
"C_3"	PX	M.R.										
"X_1"	PX	M.R.										
"X_2"	PX	M.R.										
Antibiotic peptides from:												
Bacillus licheniformis A	MPX	18.1		18.7		32.5			7.4			5.8
Bacillus licheniformis B	MPX	18.0		18.7		34.2			8.0			6.1
Bacitracin A	X	M.R.			1	1			1	1		
Bacitracin A	X	M.R.			1	1			1	1		
Bacitracin A	X	15.8		0.0	8.8	9.4	0	0	12.2	8.8‡‡	0	0
Bacitracin A	X	15.8	2.7		7.8	8.2			10.9	7.5‡‡		
Bacitracin A	X	M.R.			1	1			1	1		
Bacitracin A	X	M.R.			0.8	0.9			1.0	1‡‡		
Bacitracin F	X	M.R.			0.9	0.8			0.9	0‡‡		
Gramicidin, an average	CX	14.6					0	36.6	0.0			
Gramicidin	X							45.0				
Polypeptin	X	M.R.							1**			
Subtilin	CM	16.3	4.9			12.3	5.2		4.8			
Tyrocidine, an average	CMX	14.4					15.0	6.2	24.0			
Tyrocidine A	X	M.R.					1.0	0	3			
Tyrocidine, polypeptide from		13.7					15.3		45.9			

* L–isomer.
† D–allo-isoleucine.
‡ D–valine.
** D-isomer.
†† PHE, GLU and ornithine are D–isomer; ASP is racemic; all others are L–isomers.
‡‡ Cysteine.

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

EU	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Other Amino Acids*	*Reference*
	1†	0‡					2		Sarcosine+N-methylvaline ; 4	Sarlet, '54
	0†	1‡					2		Sarcosine+N-methylvaline ; 4	Sarlet, '54
	0†	1.9‡					1.7*		Sarcosine, 1.5 ; N-Methylvaline (L), 1.7	Brockmann, '53
	0.9†	0.9‡					1.9*		Sarcosine, 1.4 ; L-N-Methylvaline, 1.9	Brockmann, '53
	2.0†	0.0‡					2.1*		Sarcosine, 1.4 ; L-N-Methylvaline, 2.0	Brockmann, '53
	0†	1.9‡					2.1*		Sarcosine, 1.9 ; L-N-Methylvaline, 1.9	Brockmann, '53
	0†	2.1‡					1.1*		Sarcosine, 2.1 ; L-N-Methylvaline, 2.1	Brockmann, '53
		4.3		1.9	10.6		4.9			Callow, '52
		4.9		1.9	10.6		4.9			Callow, '52
	3		1	2					Ornithine, 1	Lockhart, '54
	2		1	2					Ornithine, 1	Porath, '53
.7	17.1	0	9.4	18.7	0	0	0	0	Ornithine, 7.6 ; See††	Craig, '52
.3	14.8		8.2	14.9					Ornithine, 6.6 ; See††	Craig, '53
	3		1	2					Ornithine, 1	Craig, '54 and Hausmann, '54
	1.8		0.9	1.8		0			Ornithine, 0.8 ; Allo-isoleucine, 0.5	Weisiger, '55
.0	1.8		0.9	1.9		0			Ornithine, 0.8 ; Allo-isoleucine, 0	Weisiger, '55
.3		22.2			5.1	26.1				*Cf.* B & B, '51
.0		23			5	10.0				Synge, '49
*	1*	1‡							α-γ-Diamino-butyric acid, 3	Hausmann, '52b
.5	4.0	3.3	4.1	12.2	4.8	2.3	3.3		Lanthionine, 6.4	Lewis, '51
.1		9.8	11.9	9.2		15.0	8.1	0		*Cf.* B & B, '51
	0	1	1	1			1		Ornithine, 1	Paladini, '54
.2		9.4	11.7	11.1			12.0		Ornithine, 11.9	Battersby, '52

	Method	Nitrogen	Sulfur	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*
ALFALFA								
Meal	M	2.85		5.3	2.0	5.0	2.4	2.1
Meal, an average	C		1.3	4.3	2.1	4.2		
Meal (Brome hay)	M	(2.4)		4.9	2.3	4.1		0.9
Meal (Lucerne hay)	PX	1.68		6.9	1.9	7.9	6.0	
Meal (Ranger alfalfa)	M			3.5	1.5	4.2	1.8	1.5
Meal, a mixture	X	1.19		4.6	1.5	4.5	2.6	1.1
Meal, chopped	M			4.9	1.6	6.9	3.3	1.4
Meal, 17% dehydrated	M			4.0	1.7	5.6	3.0	1.5
Meal, sulfur deficient	M	3.01		5.5	1.4	2.8	1.3	0.9
Seed (Ranger alfalfa)	M			8.6	2.2	3.9	2.4	0.7
BARLEY								
Meal	M			5.7	1.5	3.3	2.8	1.6
Meal	M	1.86		4.6	1.8	2.4		1.1
Meal	M			4.6	2.1	3.5		
Meal	X	1.53		4.8	2.0	3.4	3.1	1.0
Meal (Newal variety)	M			4.7	1.4	3.2		
Meal (Michigan winter variety)	M	4.8				4.9		0.9
Meal (Olli variety)	M				Essential amino acids in samp			
Meal, feed grade	X			5.2	2.4	3.3	4.4	1.2
Meal, malted, ground	M			4.7	1.9	4.8	3.2	1.2
Leaf meal	M	14.2		17	4.5	7.3	2.5	2.0
Leaf meal	M			7.5	2.4	5.5	4.6	2.1
Leaf cytoplasmic protein	M	14.8		16	4.4	7.7	2.5	2.2
Leaf cytoplasmic protein	M			7.5	2.4	6.0	5.0	2.3
Seedling	M	14.2		17	4.7	8.9	2.6	2.1
Seedling	M			7.5	2.5	6.7	5.0	2.2
Seedling	PX			1.7		2.0		
Isolated Seed Proteins:								
Albumin	M	15.3		13	4.5	8.3	2.6	1.4
Globulin	M	17.5		20	2.8	5.8	1.3	0.6
β-Globulin	X			2.7	1.7	2.1	8.5	3.8
Hordein	M	16.6		6	2.1	0.8	1.5	0.7
Hordein, an average	CMX	17.2	0.94	2.5	0.9	0.0	3.8	1.0
Hordein	M	15.9		12	4.3	4.8	1.9	1.1
CEREAL GRAINS, A Composite: Barley, Rye, Oats, Hard and Soft Wheat Protein, Leaf Protein	M					8.5		0.8
CLOVER								
Meal, 1st growth	PX			1.9		3.3		
Meal, 2nd growth	PX			1.6		3.1		
Meal, Ladino clover *(Trifolium repens)*	M			4.0	1.9	4.9	2.9	1.8
Meal, Red clover *(Trifolium pratense)*	M	4.52		2.1	1.9	4.1	3.0	1.8
Meal, Red clover *(Trifolium pratense)*	PX	2.45		6.5	1.8	6.7	2	
Meal, Red clover *(Trifolium pratense)* late-flowering	PX			2.0		2.1		
Seed, Subterranean clover *(Trifolium subterraneum)*	PX			11.7	3.1	9.3	2.3	
COCKS-FOOT *(Dactylis glomerata)*	PX			6.8– 8.6	1.2– 1.7	4.7 6.3		
CORN								
Meal (Mexico)	M			4.4	2.3	2.5		0.1
Meal	M			2.1	1.8	1.8		
Meal	M	2.22		4.2	2.9	2.4		
Meal	M			4.2	2.4	3.4	4.3	0.7
Meal	M	1.4				3.0– 4.2		0.5 0.6
Meal	M			5.0	2.8	2.2	4.0	0.8
Meal, an average	CM			3.9	2.3	2.1	6.1	0.8
Meal (Teosinte corn)	M	3.5				1.6 2.0		0.2 0.3
Meal (Yellow corn)	M	2.22		4.7	2.2	2.3		0.5
Meal (White corn)	M			4.5	2.7	4.5	5.6	0.8
Meal, Low-protein corn	M					3.1		09.
Meal, Low-protein corn	M			6.1	2.2	3.0	3.7	1.2
Meal, Low-protein corn	CM	1.62				3.1		0.9
Meal, High-protein corn	M					2.8		1.0
Meal, High-protein corn	M			5.3	2.2	2.4	4.1	1.0
Meal, High-protein corn	CM	2.28				2.7		0.9
Corn Grits	M			5.8	2.4	2.7	6.6	1.0
Hominy, White	M			4.2	2.0	3.9	3.6	0.8
Hominy, Yellow	M			5.3	2.3	4.1	4.0	1.0
Germ	M	3.93		8.9	2.7	5.9		0.9
Germ	M			5.6	3.0	5.3		
Germ, an average	CM		1.05	6.7	2.7	5.0	5.5	1.3
Gluten, an average	C	13.4		3.2	1.9	2.0		
Gluten (Corn gluten feed)	M			3.5	2.4	3.2	3.7	0 6
Leaf Meal	M			3.9	1.3	3.2		1 3
Pollen, mixed	M			5.7	2.4	6.3		1.3
Pollen, sweet corn	CM			4.7	1.5	5.7		1 6
Pollen, an average	M			5.3	2.5	6.4		1 4

* For other values, see also *FOODS;* Animal Feeds.

REALS, GRAINS, GRASSES, FEEDS* AND FORAGE

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

S	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.6	4.9	6.5	10.2	4.8	6.1	7.9	10.6	4.9	6.2	5.1		Mertz, '52b
													Cf. B & B, '51
	0.5		4.3	6.6	5.1	4.7							Chance, '53
		6	10	9			11	9	8	5.9	2		Koloušek, '54 & '55b
	1.3	3.9	5.0	7.9	4.2	4.9	7.9	12.8	3.6	6.3	4.5		Singleton, '52
	1.6	3.6	3.9	6.9	4.1	5.2	10.0	9.1	4.9	6.0	5.8		Dustin, '53a
	0.8		4.0	7.1	6.2	4.6							Williams, H.H., '55
	0.4		3.8	6.9	4.7	4.3							Williams, H.H., '55
	0.8	3.2	3.0	5.1	2.9	3.3	2.9	33.2	2.7	3.0	2.6		Mertz, '52b
	1.3	3.8	3.8	7.4	3.7	4.4	11.0	8.2	4.6	4.0			Singleton, '52
	3.5	5.3	3.2	6.8	4.8	3.8	22.0	2.5	2.9	4.1	4.8		Ågren, '49a
	1.0		3.6	7.7	4.6	5.2							Horn, '50
	1.4		5.0	6.8	3.8	5.2							Horn, '53
	1.6	3.6	3.7	6.8	3.3	4.8	20.8	5.9	4.3	4.4	9.3		Dustin, '53a
	1.2		3.0	6.6	4.1	4.9							McElroy, '49
	1.4		3.6	5.4	5.3	5.4	12.0						Raber, '52 & '53
own on sulfur-deficient soil													Renner, '53
	1.0	4.0	3.1	8.8		3.8	23.0	6.4	3.7		10.2		Kraut, '55
	1.1		3.1	6.6	4.6	4.3							Williams, H.H., '55
	1.5	3.7	4.5	6.5	5.8	5.6	9.2	7.4	7.3	9.0	4.5		Folkes, '51
	2.2	4.0	5.4	8.7	7.1	6.7	13.4	10.0	5.6	8.1	5.3		Yemm, '53
	1.5	3.8	4.3	6.4	4.5	5.3	8.9	6.9	7.2	8.5	4.4		Folkes, '51
	2.4	4.2	5.4	8.9	6.3	6.6	13.6	9.7	5.7	8.0	5.4		Yemm, '53
	1.4	3.9	4.5	6.8	5.1	5.7	9.0	7.4	7.8	9.3	4.4		Folkes, '51
	2.0	4.2	5.4	9.0	6.8	6.8	13.4	10.0	5.9	8.4	5.1		Yamm, '53
		5.5		10.9		6.7	17.6	7.7		5.4	2.0		Smith, A.M., '51c
	1.5	4.3	3.6	6.0	4.3	6.1	9.2	8.4	7.0	7.5	4.4		Folkes, '51
	0.8	3.6	2.2	4.1	2.0	3.7	6.3	5.1	9.8	0.6	2.5		Folkes, '51
	3.2	3.7	5.9	10.9	3.2	9.5	17.8	8.8	4.2	6.5	13.3	0 ?	Jensen, '52
	0.7	3.1	1.8	4.4	3.5	3.5	22.2	1.3	1.6	2.1	14.8		Folkes, '51
	2.1			7.0		2.0	38.4	1.2	0	1	6.0		*Cf.* B & B, '51
	1.1	4.2	3.1	5.8	3.5	4.9	11.6	4.7	5.2	6.6	6.6		Folkes, '51
	1.6		4.8	6.7	5.8	5.9	11.7						Reber, '53
		7.1		14.5		9.1	13.1	9.4		6.3	3.7		Smith, A.M., '51c
		7.8		13.0		9.1	13.3	7.0		6.7	3.0		Smith, A.M., '51c
	1.1	4.0	5.2	8.8	5.0	5.1	6.0	13.8	4.0	6.3	5.8		Singleton, '52
	0.7		2.5	8.1	4.8	5.6			5.6	4.8			Ågren, '52
		8	10	9			11	8	5.7	7.0	1		Koloušek, '54 & '55b
		6.7		14.0		7.2	14.8	8.7		4.9	2.1		Smith, A.M., '51c
	1.5		1.8	4.6	2.7	4.0	23.2	8.5	3.4	4.1			Holmes, '53
													Waite, '53
	1.9		4.7	19.6		5.4							Massieu, '49
	1.6		3.5	9.8	2.7	4.3							Jackson, '53
	2.0		3.7	12.7	4.1	4.9							Horn, '49 & '55
	1.3		3.6	10.8	4.3	4.3							Williams, '55
	1.7–1.7												Melkus, '53
8	2.3		4.7	11.9	4.1	4.7							Edwards, C.H., '55
3	2.7	4.8	3.6	14.8	4.1	4.5	24.5		4.3		5		*Cf.* B & B, '51
	2.2–2.5												Melkus, '53
	1.4		3.9	14.4	4.4	5.3							Horn, '50
	0.9		3.8	10.7	4.4	4.5							Williams, H.H., '55
	1.2												Miller, R.C., '50
9	2.1	4.0	4.9	9.3	3.6	7.5	15.1	12.5	3.2	9.3	7.1		Sauberlich, '53
5													Flynn, '54
	1.4												Miller, R.C., '50
9	1.9	4.5	4.7	12.6	3.9	7.1	15.6	12.1	2.9	10.6	9.6		Sauberlich, '53
3													Flynn, '54
2	1.9		4.3	11.6	6.1	4.7							Edwards, C.H., '55
	0.7		3.2	8.6	3.6	3.9							Williams, H.H., '55
	0.9		3.7	8.3	4.4	4.1							Williams, '55
	1.4		4.4	7.5	3.7	5.7							Horn, '49 & '50
	1.5			7.1	4.2	5.3	15		5.5				Dunn, '49d
.2	1.5		4.2	7.1	4.2	5.3							*Cf.* B & B, '51
													Cf. B & B, '51
	1.3		3.4	10.9	5.1	4.5							Williams, H.H., '55
	2.8		3.3	6.9	3.6	4.8							Kelley, '53
	1.8		4.0	6.7	4.5	5.7							Weaver, '51
.6	1.7		4.6	5.6	4.7	6.0	9.1						Sarkar, '49a
	1.9		4.1	7.1	5.1	5.8							Weaver, '51

	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY
Isolated proteins:								
Zein	C	16.13			0.8			
Zein	C	15.7						
Zein	M	16.0		2.0	0.8	0.2		0
Zein	X			1.8		0.0	6.6	
Zein, an average	CM	16.1	0.52	1.6	1.2	0.1	5.0	0.1

REALS, GRAINS, GRASSES, FEEDS* AND FORAGE (Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

S	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
													Vickery, '44
		5.2	3.0										Neidig, '52
	1.4		2.6	21.1	5.0	4.0							Horn, '50 & '49
	1.5	5.9	3.3	26.9		4.0	31.8	5.4	1.4	10.3	10.6	0	Thompson, '52
	2.1	7.7	2.7	22.3	7.3	2.6	28.4	3.3	0.4	9.9	10.0		*Cf.* B & B, '51

	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY
Feeds, miscellaneous								
Brewers' Dried Grains	M			4.6	1.8	3.3	4.1	1.3
Corn Distillers' Dried Grains	M			3.5	2.1	3.2	3.0	0.6
Distillers Dried Grains	M			3.6	2.0	3.2	3.1	0.8
Distillers Dried Solubles	M			3.8	2.1	4.3	3.0	0.8
Fenugreek *(Trigonella Foenum-Graecum)*								
Seed protein		13.88		8.0	1.1	8.0	3	
Fescue								
Extracted protein	PX			6.6- 8.0	1.2- 1.6	5.8- 6.9		
Meadow fescue *(Festuca pratensis)*	M	3.18		2.7	1.7	5.2	2.4	2.5
Grasses, composite samples								
An average, 7 grasses	PX					3.3		6.6
An average	CM	13.9	1.2	6.3	1.7	4.8	4.9	2.1
Various, a range	M				1.5 2.1	2.1 4.5	2.3- 4.1	1.3 2.1
Hay, see Alfalfa								
Job's-Tears *(Coix lachryma-jobi)*	M			3.5	2.2	2.5		0.5
Millet								
Meal (native, 2 varieties)	M			3.6	2.0	2.7		1.8
Meal (cultivated, 3 varieties)	M			4.7	2.1	2.6		2.3
"Cumbu," India *(Pennisetum vulgare)*	M	1.81			3.8	1.8		1.9
"Ragi," India *(Eleusine coracana)*	M	0.59			3.4	3.0		1.6
"Ragi," India *(Eleusine coracana)*	M	1.28		1.5	0.4	1.8		1.0
Finger millet	M	1.26				2.6		1.3
Oat								
Meal	C	(16.0)		11.3	4.7	7.6	3.0	1.2
Meal				6.3	2.2	4.3	3.6	1.4
Meal	M	2.73		7.4	2.2	3.0		1.1
Meal	M	(16.0)		6.7	1.5	3.4	4.5	1.3
Meal	M					3.4		1.2
Meal	M			5.7	1.7	4.5	3.8	1.3
Meal	M			6.8	2.1	3.9		
Meal (Victory)	M			6.1	1.3	3.3		
Meal (Wintok)	M					5.9		1.0
Meal (Wintok)	M	3.8				6.0		0.8
Meal, an average	CM			4.9	2.1	3.1	4.5	1.3
Chaff	PX			10.7	3.1	7.1	3.1	
Oat Mill Feed	M			4.1	1.1	3.5	2.8	0.7
Mash oats, pulverized	M			5.4	1.6	4.1	3.4	1.3
Seeding oats	PX			1.2		1.2		
Rice								
Meal	M	1.26						
Meal, Ceylon	M					3.5		1.2
Meal, India	M	1.34			3.9	2.2		1.2
Meal, Mexico	M			6.0	2.0	3.1		1.4
Meal, white rice	M	1.26		8.8	2.3	2.8		0.1
Meal, white rice	M			8.3	2.2	3.7		
Meal, brown rice	M			8.4	2.4	3.9		
Meal, brown rice	M			8.6	2.5	4.5	5.8	1.1
Meal, an average	CM			7.1	2.2	3.4	5.7	1.1
Isolated fractions and proteins:								
Albumin (from polished, nonglutinous rice)	M	12.31		9.5	2.9	8.7	4.5	1.8
Germ								0.7
Germ	M	14.9		10.5	2.9	11.5	6.2	1.8
Globulin (from polished, nonglutinous rice)	M	14.32		2.5	0.7	0.9	6.8	1.1
Glutelin							5.8	1.8
Glutelin (from polished, nonglutinous rice)	M	14.25		9.0	3.2	3.3	3.0	1.3
Prolamine	M	13.9		5.9	1.2	0.6	9.6	1.2
Rye								
Meal	M	1.98		5.4	2.2	3.3		0.8
Meal (Annual rye grass)	M	4.2				5.6		1.0
Meal (Balboa rye)	M	4.3				5.3		0.8
Meal (Balboa rye)	M					5.0		0.8
Extracted protein	PX			7.2 8.2	1.1 1.8	5.3 6.4		
Seedling (Italian rye)	PX			1.5		1.8		
Sorghum								
Meal	M	1.93				1.5		0.9
Meal, average of 4 varieties	M			3.5	2.2	2.1		1.1
"Cholam" India *(Sorghum vulgare)*	M	1.21			3.4	1.6		1.2
Yellow Milo Sorghum, ground	M			3.2	1.8	2.6	4.2	1.0
Kafirin (an isolated protein)	C	16.4						
Timothy								
Extracted protein	PX			6.2 7.5	1.3 1.7	5.6 6.5		
Meal	PX	1.64		1.8	1.8	[illegible]	[illegible]	

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.2		3.2	8.3	5.4	5.4							Williams, H.H., '55
	1.2		1.0	12.5	3.5	3.5							Williams, H.H., '55
	1.3		3.4	9.9	5.7	4.2							Williams, H.H., '55
	1.3		3.4	9.0	4.2	5.1							Williams, H.H., '55
		6	5	11			9	9	9.5	5.9	1		Koloušek, '55a
													Waite, '53
	0.8		3.4	8.0	4.9	5.0			5.7	5.4			Agren, '52
		4.8	5.8	8.3	5.1	6.2			5.8	6.0	4.7	0	Kemble, '54
0	2.3		4.7	8.4	5.7	6.8	12.3	5.3					*Cf.* B & B, '55
	0.8–2.1												Armstrong, '51
8	3.1		4.2	2.37	7.2								Adrian, '55
3	2.4		2.7	16.8	5.0	4.4							Adrian, '55
3	2.6		4.9	17.4	4.3	5.7							Adrian, '55
	1.8			9.5	5.9	6.2							Balasubramanian,'52ab
	1.5			9.5	6.4	6.7							Balasubramanian,'52ab
			0										Lal, '50
	3.2		3.9	8.9	4.7	8.5							Baptist, '54a
.1													Yaichnikov, '54
.5	3.1	2.9	2.1	8.1	5.4	3.8	18.0	4.5	3.1	4.1	4.1		Agren, '49a
	1.1		3.6	7.7	4.5	5.3							Horn, '49 & '50
.8	1.5	4.5	3.4	5.7	5.1	6.6	20.0	3.7	4.1	6.2	5.7		Heathcote, '50b
	1.2			7.0									Frey, '52
	1.0		3.1	7.1	4.4	4.4							Williams, H.H., '55
	1.5		3.4	7.4	4.4	5.7							Horn, '55
	1.2		3.0	7.0	5.0	5.7							McElroy, '49
	1.7		5.0	6.7	4.9	5.6	11.0						Reber, '53
	1.0		6.5	7.6	5.6	4.9	8.1						Reber, '52
.0	1.9		3.2	7.6	4.6	5.4							*Cf.* B & B, '49a
	2.0		3.7	8.2	6.0	6.6	20.5	10.0	6.9	4.8			Holmes, '53a
	0.4		2.6	5.8	3.4	3.7							Williams, H.H., '55
	0.6		2.9	6.7	4.3	4.7							Williams, H.H., '55
		4.7		9.2		5.9	17.2	9.2		4.8	2.9		Smith, A.M., '51c
					4.2								Horn, '49
	1.7		3.5	8.1	4.5	6.7							Baptist, '54a
	1.6			8.0	5.9	6.1							Balasubramanian,'52ab
	1.8		4.3	9.2		6.4							Massieu, '49
	1.4		3.6	8.6	4.4	6.4							Horn, '50
	2.3		3.4	7.8	4.5	6.2							Horn, '55
	2.1		3.6	7.9	4.6	6.2							Horn, '55
0.9	2.2		3.2	9.0	4.5	6.4							Edwards, C.H., '55
1.2	1.4		3.4	8.2	5.2	6.2							*Cf.* B & B, '51
3.6	2.5	9.0	4.3	12.7	7.7	10.1	22.8	7.0	5.7		7.6		Tamura, '52
													Cf. B & B, '51
1.1	2.8	6.2	14.7	5.6	4.2	6.3	8.4	8.8	6.9		5.8		Kik, '54
1.4	5.4	7.8	1.8	6.6	1.8	4.8	23.6	16.5	5.7		6.1		Tamura. '52b
1.2	3.4												*Cf.* B & B, '51
0.8	1.5	7.0	3.4	10.4	5.5	9.1	23.4	9.4	5.2		4.3		Tamura, '52b
0.7	3.9	8.0	1.8	12.5	5.0	8.2	34.8	7.7	4.4		7.3		Tamura, '52b
	1.1		4.0	6.7	4.1	5.0							Reber, '52
	1.1		7.8	8.8	6.2	5.0	8.7						Reber, '52
	1.2		8.7	9.5	6.4	5.3	8.7						Reber, '53
	1.7		5.3	7.8	4.8	6.2	11.0						Waite, '53
		5.0		11.9		7.5	18.0	9.3		5.1	2.1		Smith, A.M., '51a
	1.4		3.3	13.1	4.4	7.0							Baptist, '54a
1.6	2.8		3.7	25.0	5.0	4.9							Adrian, '55
	1.6			13.0	6.1	5.9							Balasubramanian,'52ab
	0.5		3.2	13.2	5.2	4.7							Williams, H.H., '55
				15		4							*Cf.* B & B, '51
													Waite, '53
		3	10	16		8	8	5.6	8.5	1			Koloušek, '54 & '55b

	Method	Nitrogen	Sulfur	ARG	HIS	LYS	TYR	TRY	PH
WHEAT									
Whole wheat	M	(16.0)							
Whole wheat	M	3.07		4.5	2.0	2.5		0.8	4.
Whole wheat	M			4.2	2.1	2.7			4.
Whole wheat	X			3.6	1.3	2.5	3.5	1.2	4.
Whole wheat, ground wheat	M			4.4	1.7	3.0	3.7	1.4	3.
Whole wheat, new crop plate		2.27		4.5		2.0			
Whole wheat, an average	CM			3.5	1.9	2.5	3.5	1.0	4.
Whole wheat, an average of 13 Dutch var.	C								
Whole wheat, India	M				2.2	1.2		1.1	5.
Whole wheat, an average of 7 U.S. var.	M					3.6			
Whole wheat, an average of 10 U.S. var.	C								
Whole wheat (Clarkan)	M					4.9		0.8	4.
Whole wheat (Clarkan)	M	3.8				6.1		0.9	4.
Whole wheat (Marquis)	M			4.5	1.6	2.2			5.
Whole wheat (Manitoba, Tough No. 2)		2.43		4.3		2.0			
Whole wheat (N.S.W.Australia)		1.81		4.5		2.2			
Whole wheat (Red Dog)	M			5.7	2.0	3.5	2.9	1.4	2.
Whole wheat (Tenmark)	M	4.6				6.3		1.0	4.
Whole wheat (Tenmark)	M					6.0		0.9	3.
Whole wheat (Thatcher)	M				Essential amino acids of sampl				
Whole wheat (White English)		1.58		5.1		2.4			
Whole wheat (Winter Wheat)	C ?			3.2– 4.1	1.4– 1.9	1.9– 3.3	3.7 4.1	1.4 2.5	
Gelatinized wheat flour	M			2.6	2.4	1.8			4.
Isolated fractions and proteins from wheat:									
Albumin		17.1		8.1				3.0	
Bran	C	(16.0)		9.2	2.5	2.1	3.2	1.9	
	M			6.3	2.1	4.3	2.9	1.5	3.
Bran, an average	CM			6.4	1.9	3.8		1.2	2.
Bran globulin	M	17.7		12.0	1.7	3.7		0.8	3.
Germ	M			5.9	2.0	6.7	3.3	1.3	3.
Germ	M	6.5		6.9	2.1	6.2		0.7	3.
Germ, an average	CM		0.50	6.0	2.4	5.5	3.8	1.0	3.
Germ, defatted	M			5.4	2.1	5.1			2.
Germ, defatted and toasted	M			5.4	2.3	4.3			3.
Gliadin	C	17.66			1.6				
Gliadin	M			2.7	1.9	0.6			
Gliadin	X	(17.1)							
Gliadin, an average	CMX	17.6	1.01	2.6	1.8	0.7	2.9	0.8	5.
Globulin		18.6		14.7				0.9	
Gluten	CX				1.4				5.
Gluten	M			3.7	2.0	2.0	2.8	0.8	5.
Gluten	M			3.8	1.9	1.4		1.1	5.
Gluten	M			3.3	1.9	1.4	3.4	0.9	4.
Gluten	M	17.5		4.3	2.1	1.6	2.8	1.0	4.
Gluten	M	15.4							
Gluten	M			3.7	2.2	1.9	3.2	0.9	5.
Gluten, an average	CM	13.5		2.9	1.6	1.5		1.1	
Wheat Standard Middlings	M			5.6	2.0	4.2	2.8	1.3	3.
Crystalline peptide from wheat	C	17.4	4.1				3		

COMMENTS—CEREALS, GRAINS, GRASSES, FEEDS AND FORAGE

Corn: Frey (1951) has reported that as the protein content of certain strains of corn increased, the increase is due primarily to zein. This results in a corn containing proteins lower biological value for monogastric animals. However, Mitchell (1952) points out th in practical rations corn of higher protein content actually reduces the quantity of supple mentary (soybean) protein needed. That is, the advantages of high protein corn overweig the loss in biological value. "Teosinte," a high protein type corn, is deficient in lysine an tryptophan even when fed to rats at a 15 per cent protein level (Yang, 1955).

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
							31.3						Mulinos, '51
	1.0		3.0	5.9	3.5	4.1							Horn, '49 & '50
	1.4		2.9	6.6	4.2	4.3							Horn, '55
0	0.7	4.7	2.5	9.3		2.9	29.3	3.8	3.4	3.0	10.3		Kraut, '55
	0.7		2.8	6.3	4.7	4.0							Williams, H.H., '55
						4.4							Price, '50
6	1.6	2.8	2.8	7.0	4.8	4.3	29.0						*Cf.* B & B, '51
4– 5													Wöstmann, '50
5	1.5			6.5	3.5	4.0							Balasubramanian, '52ab
5	1.6						36.2						Miller, B.S., '50
3 - 9													Wöstmann, '50
	1.5		4.3	6.7	4.4	5.9	10.3						Reber, '53
	1.3		7.3	8.5	5.5	5.7	7.6						Reber, '53
	1.2		2.7	6.4	4.5	4.4							McElroy, '49
						4.2							Price, '50
						4.6							Price, '50
	0.6		2.8	6.5	3.2	3.8							Williams, H.H., '55
	1.1		7.9	8.9	5.8	5.3	8.4						Reber, '52
	1.8		4.2	6.2	4.0	5.3	9.4						Reber, '53
rown on sulfur-deficient soil													Renner, '52
						4.8							Price, '50
2– 9													Kizima, '55
	0.95			7.0	4.7	4.3	31		3.3				Dunn, '49
													Pence, '53
.0													Yaichnikov, '54
	0.6		2.6	5.5	3.8	3.9							Williams, H.H., '55
.5	1.1		2.4	6.5	4.5	4.1							*Cf.* B & B, '51
	0.9		3.2	5.7	3.4	6.0							Horn, '50
	1.0		3.4	5.5	4.5	4.5							Williams, H.H., '55
	1.3		4.0	6.0	3.6	4.8							Horn, '49 & '50
.1	1.4		5.0	6.7	3.9	4.8							*Cf.* B & B, '51
	1.2			6.0	3.7	4.4	13		5.7				Dunn, '50d
	1.3			5.9	4.1	4.8	16		5.2				Dunn, '50d
													Vickery, '44
			1.9				42.0	2.1					Yemm, '53
							22.2						Barker, '51
.1	1.8	3.8	1.9	6.1	4.7	2.4	40.5	1.6		1.9	12.1		*Cf.* B & B, '51
													Pence, '53
	1.6			7.6	3.6	4.0							Miller, S., '52
.4	1.7		2.8	7.2	4.5	4.3	35.0	3.8	3.3		14.1		Rutgers Bull., '50
	1.7		2.6	6.3	4.8	4.6							Miller, '52
.2	1.3		2.1	5.6	3.6	3.5	28.6	2.7	2.8	1.7	10.7		Rice, '50
.7	1.7	4.3	2.4	7.0	4.2	4.3	32.5	3.4	3.2	2.0	11.6		Pence, '50
									3.4				McCoy, '53a
	1.6		2.7	7.0	4.4	5.2							Williams, H.H., '55
.5			2.5				26.8	9.6	7.2	2.7	8		*Cf.* B & B, '51
	0.9		3.1	6.7	4.4	4.0							Williams, H.H., '55
.2													*Cf.* B & B, '51

Wheat: The nutritional value, i.e., essential amino acid content of wheat may be affected by environment (Miller, B.S., 1950), and total protein content (Price, 1950; *cf.* Moyer, 1952). As the total protein of wheat is increased, the content of certain essential amino acids is decreased.

General comments: A number of reports have appeared on the nonprotein amino acids in various plants and the isolation therefrom, of amino acids which do not occur in the usual tissue protein (Bathhurst, 1954; Esh, 1954; Grobbelaar, 1955; Rabideau, 1952; Thompson, 1953; *cf.* Block, Durrum and Zweig, 1955).

	Method	*Nitrogen*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*	*CYS*	*MET*	*SE*
AGATI LEAF, Meal (India)	M	5.7	5.8	1.9	4.0		2.9	6.0	1.5	1.4	
AMARANTH LEAF *(Amaranthus gangeticus;* India*)*, Meal	M	4.76	4.2	2.2	4.4	3.3	1.9	3.0	0.7	1.2	
APPLE											
Dried tissue	M		3.5	1.4	4.0		0	3.3		1.7	3.
Plant juice protein *(Malus sylvestris)*	M		7.9	1.2	1.9	4.3		4.5	1.1	3.6	3.
BEET *(Beta vulgaris)*											
Pulp	X		2.7 2.9	0.9 1.3	1.8– 2.0	2.8	2.3	1.1	0.4 0.8	0.0– 0.4	3. 3.
Pulp, dried	M		3.7	2.2	7.7	4.6	1.0	3.0		0.1	
Leaf meal	M		4.1	1.3	5.4		1.2	5.8		1.7	
Leaf protoplasts	M		5.9	1.9	5.6		1.7	6.5		1.6	
Plant juice protein	X		7.1	1.2	2.1	5.8		5.8	1.5	4.3	5.
BROCCOLI											
Heads	M		6.5	2.1	2.7		1.2	4.0		1.2	
Leaf meal	M		4.8	1.5	4.5		1.4	6.0		1.8	
Leaf meal, material soluble in formic acid	M		6.0	2.2	5.5			7.5		2.0	
Leaf protoplasts	M		5.2	1.8	5.3		2.3	7.8		1.8	
Stalks	M		5.3	1.8	5.1		1.0	3.6		1.3	
CABBAGE, Meal	M		7.8	1.6	3.3	2.1	0.8	3.2	1.6	0.5	
CARROT											
Meal	M		3.6	1.5	3.1		0.6	4.1		1.0	
Leaf meal	M		4.3	1.9	4.5		1.4	6.5		1.7	
Leaf meal, material soluble in formic acid	M		5.7	2.1	5.4			7.7		3.4	
Leaf protoplasts	M		6.9	2.0	5.5		2.2	8.4		1.8	
CASSAVA *(Manihot esculenta;* Bitter cassava, Manioc*)*											
Flour	M	as ratio %	4.6	1.7	5.2	1.9	0.6	2.9	0.5	1.4	3.
Flour	X	0.32	3.7	1.2	3.5	1.5	0.5	2.2	0.6	1.0	1.
CELERY, Leaf meal	M		4.0	1.5	2.4		1.3	4.5		2.2	
COLLARD GREENS	M		7.4	2.2	4.7	3.9	1.2	2.3	1.5	1.1	
CUCUMBER											
Globulin	M	(18.6)					1.7	5.6			
Seed globulin	X	18.5	13.6		2.6	3.3		8.6	1.0	1.9	4.
KALE	M		5.1	1.6	3.1		1.1	4.4		0.9	
MUSHROOM, Mycelium	PX		1.0		0.6	0.8		2.5			1.
MUSTARD GREENS	M		7.3	1.8	4.8	5.3	1.6	3.2	1.5	1.0	
OKRA											
Meal	M	3.26	2.5	1.1	2.7		1.2	3.9	0.8	1.2	
Meal	M		6.4	1.8	4.5	4.4	1.4	3.6	1.1	1.7	
ORANGE											
Navel variety	PX	15.37			5.3	8.0		7.8			4.
Valencia variety	PX	15.12			7.3	1.4		3.9			3.
POTATO											
Meal, white	M		7.1	1.2	3.7	2.5	1.0	3.6	0.6	2.5	2.
Meal, white	M		5.0	1.5	5.5		0.1	4.7		1.5	
Meal white	CMPX		4.4	1.7	5.0		0.8	5.4	1.6	1.6	
Meal white, an average	CMX		4.6	2.0	6.7		1.6	5.6	1.3	2.1	
Meal, sweet potato	M		5.2	2.4	5.5		2.1	6.1		2.6	
Potato protein without alcohol extractive	X	(16.0)	6.0		8.0	3.9		12.7		1.5	3.
Tuberin	CMPX		6.0	2.2	7.7		1.6	6.6	2.1	2.3	
PUMPKIN											
Seed globulin	M	18.5	13.8–			3.8	1.5	6.2			
Seed globulin	C	18.55		1.8							
RHUBARB											
Leaf meal	M		4.7	1.9	5.4		1.6	6.1		1.0	
Leaf meal, material soluble in formic acid	M		6.3	2.4	5.4			6.9		2.1	
RUTABAGA			5.4	1.7	5.6			5.8		1.6	
SPINACH											
Meal	M		6.4	2.8	7.6		1.9	5.4		2.0	
Meal	M		4.4	1.3	4.7		1.1	4.7		2.3	
Meal, material soluble in formic acid	M		5.4	1.8	5.8			5.8		1.8	
SQUASH											
Seed meal	C	16.34									5.
Seed globulin	X	18.5	13.2		3.5	3.2		7.2	0.7	2.2	4.
TOMATO, Pomace, ground	M		5.3	1.9	7.2	4.1	0.9	3.8		0.2	
TURNIP											
Meal	M		4.5	1.4	3.0		1.3	5.3		2.2	
Greens	M		6.3	1.7	7.7	3.7	1.9	4.9		0.3	
Greens	M		7.7	2.5	4.1	3.6	1.9	4.7	1.5	2.8	
YAMS	M		3.4	1.6	4.1	4.5	1.8	5.4	1.6	1.1	
WATERMELON											
Seed globulin	M	(18.6)					1.6	6.6			
Seed globulin	C	18.64		2.0							

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* Includes glutamine.
† Includes asparagine.

HR	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Other Amino Acids*	*Reference*
4.8	8.9	6.2	6.8								Baptist, '54b
2.4	6.5	5.1	5.0								Deshpande, '54
3.1	5.2	5.3	3.7	6.1	4.3	2.9		4.2			Davis, '49
3.1	28.4	6.2	8.0	32.3*	15.5†	3.3	3.7	4.5			Mansford, '54
2.2	1.8	1.5	2.1–	48.8–	7.3–	1.7–	2.1–	1.3–	0–		Bigwood, '54
3.3	2.0		2.4	72.8	8.0	1.9	3.7	2.0	2.9		
4.1	6.0	4.1	4.8								Williams, H.H., '55
3.8	6.4	4.2	5.1								Kelley, '53,
4.9	8.6	5.5	6.3								Kelley, '53
5.4	16.2	3.2	8.3	35.6*	13.7†	4.0	6.9	5.9		Glutamic acid and aspartic acid contain glutamine & asparagine, resp.	Mansford, '54
3.7	4.8	4.0	5.7								Hirsch, '52
3.3	6.4	3.2	4.5								Kelley, '53
5.0	9.2	5.3	6.4								Kelley, '53
4.4	8.9	4.7	5.9								Kelley, '53
3.9	4.5	3.7	5.6								Hirsch, '52
2.6	4.5	2.6	2.8								Edwards, C.H., '55
4.0	5.6	4.4	5.7								*Cf.*, B & B, '51
4.4	7.1	4.5	5.5								Kelley, '53
5.0	10.6	5.5	7.0								Kelley, '53
6.1	10.7	6.3	7.2								Kelley, '53
3.8	4.8	3.2	4.7	18.8	7.2	6.7	11.1	2.9		Ornithine, 14.7	Bigwood, '53b
2.1	2.9	2.0	2.6	12.8	4.5	2.4	4.7	1.5		Ornithine, 10.5	Close, '53a
3.4	6.8	3.9	4.8								Kelley, '53
1.5	3.7	1.4	3.7								Edwards, C.H., '55
	7.8		6.0								*Cf.*, B & B, '51
2.9	11.1		4.7	18.5	8.1	4.2	4.4	4.2			Thompson, '52
3.5	6.5	3.4	4.6								Kelley, '53
2.3			2.3	6.9	4.6	1.1	2.6				Block, unpub.
2.6	2.7	3.3	4.7								Edwards, C.H., '55
3.8	6.2	4.2	5.9								Baptist, '54b
3.6	5.7	3.8	4.6								Edwards, C.H., '55
2.6	6.2		4.2	5.3	10.4	5.9	5.4	3.0		Ornithine, 14.5	Wedding, '54
1.6	7.1		6.6	12.7	13.8	8.8	6.6	6.5		γ-Aminobutyric acid, 11.2	Wedding, '54
2.5	4.6	5.9	4.3	7.4	11.5	1.9	6.1	3.0			Ågren, '49a
3.4	4.8	4.3	5.8		8.0						Hirsch, '52
	11.3		4.8								Chick, '49
5.3	9.6	5.0	5.4								*Cf.*, B & B, '51
3.6	5.6	5.1	9.3								Hirsch, '52
3.2	17.6		7.7	14.6	12.0	5.6	6.2	3.5			Thompson, '52
	17.5		6.1								Chick, '49
	6.9	4.6	5.6								*Cf.*, B & B, '51
											Vickery, '44
4.0	8.4	4.0	5.3								Kelley, '53
4.5	9.8	5.0	6.6								Kelley, '53
4.2	7.2	4.7	6.0								Kelley, '53
5.5	8.1	5.4	7.1								Hirsch, '52
3.9	6.8	3.6	5.0								Kelley, '53
4.6	8.2	4.2	6.0								Kelley, '53
3.0											Neidig, '52
2.6	11.5		4.8	20.9	5.9	4.8	4.9	4.7			Thompson, '52
3.4	7.6	3.3	4.4								Williams, H.H., '55
4.0	6.8	3.9	4.8								Kelley, '53
4.8	9.3	4.4	6.4								Williams, H.H., '55
4.3	5.6	2.6	4.3								Edwards, C.H., '55
3.4	5.7	4.0	5.3								Edwards, C.H., '55
											Cf., B & B, '51
	6.5		5.5								Vickery, '44

	Method	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*
ALMOND									
Amandin	C	19.0		11.2	1.6	0.6	0.9	1.2	
Amando-albumin	C	18.2		9.5	1.9	5.0	2.7	1.2	
Amando-globulin	C	18.2		8.5	1.8	3.3	3.1	1.5	
BEANS, see LEGUMES									
BRAZIL NUT, Meal	M	9.03		13.5	1.9	3.2		1.3	3.6
COCOA									
"Pataste," Mexico *(Theobroma bicolor)*	M	7.26		9.3	1.5	4.9		1.3	5.8
CAROB BEAN, Gluten	M			12.8	2.5	4.3	2.2	0.6	2.7
COCONUT (see also PALM)									
Meal	M			10.5	2.1	3.7			4.0
Meal (copra)	M			11.0	1.6	2.6	4.0	0.8	3.9
Meal (copra cake)	CPX	3.3		7.0	2.9	4.8		1.6	5.2
Meal (oil meal)	M			10.8	1.6	3.0	1.9	1.0	3.9
Globulin	C	18.5							
Globulin	M	17.4		15.0	1.4	4.0		0.6	4.7
COFFEE BEAN, Meal (Santos var.)	X								6.3
COTTONSEED									
Meal	C	(16.0)		10.4	1.7	3.9	0.9	1.4	
Meal	M	10.36		11.9	2.5	4.5		1.1	5.3
Meal	M			12.4	2.9	4.9			5.4
Meal	M			11.6	2.9	4.2		1.2	5.6
Meal	PX			10.9	2.8	3.9	2.4	0.9	4.9
Meal	X	9.36		12.3	2.6	4.3	3.1	1.2	5.8
Meal (cake)		(16.0)		20.5	3.7	5.8	1.5	1.8	
Meal (oil meal)	M			9.0	2.5	4.7	3.0	1.3	5.2
Meal, an average	CM	9.7		10.0	2.6	3.7	2.1	1.4	5.8
Globulin	M	18.0		13.5	3.1	3.8		1.2	7.5
Globulin, an average	C			12.2	2.8	4.1	2.9	1.5	6.4
FILBERT NUT									
Meal, oil-free (Barcelona var.)	M			15.6	1.8	2.8	3.7	1.1	3.6
Meal, oil-free (Du Chilly var.)	M			14.3	1.8	3.1	3.8	1.1	3.5
Globulin (Barcelona var.)	M	(16.0)		14.4	2.4	2.1	3.3	1.5	3.8
Globulin (Du Chilly var.)	M	(16.0)		14.5	2.0	2.0	3.2	1.5	3.5
FLAXSEED, see LINSEED									
GUAR SEED	PX	8.04		2.3	0.9	5.2		1.7	2.7
HEMPSEED									
Meal	C							1.0	
Edestin	C	18.69			2.2				
Edestin	M	18.55		14.2	2.2	2.8		1.1	4.7
Edestin	M				2.7				4.5
Edestin	M			15.4	2.5	2.4			
Edestin	X				1.2				5.0
Edestin	X	18.6		12.7		1.7	4.0		4.0
Edestin, an average	CMX	18.3		13.6	2.5	2.4	3.5	1.3	3.6
LEGUMES									
Aconite Bean, India *(Phaseolus aconitifolius*, Moth bean*)*	M	3.91			3.3	4.6	5.6	0.7	4.5
Adzuki Bean, globulin *(Phaseolus angularis)*				5.1	1.7	4.0			
Bean (see also specific varieties)									
Meal	CM	(16.0)		5.2	2.5	6.9		0.8	5.0
Phaseolin	M	16.1		5.9	2.2	7.1		0.5	8.0
Phaseolin (Ukrainian beans) range		(16.0)							
Black Gram, India *(Phaseolus mungo)*	M	4.78				6.8		1.1	5.9
Black Gram, India *(Phaseolus mungo)*	C	3.89				5.1		1.3	4.0
Black Gram, India	M	4.05		5.7	2.7	6.0		0.5	5.4
Black-Eyed Pea (Vigna sinensis, Cow-pea*)*									
Meal	M	4.15		7.5	2.9	6.6		0.9	5.2
Meal	M			7.8	3.0	9.6	4.7	1.4	5.7
Meal	M			7.6	3.0	7.1			5.8
Meal (dry)	M			6.4	3.2	6.3		1.0	5.1
Meal (fresh)	M			6.1	3.3	6.3		1.0	5.5
Meal (cow-pea, India)	M	3.94		6.8	3.1	6.2		0.6	5.2
Chick-Pea (Cicer arientinum, Gram, Bengal gram*)*									
Meal, Bengal gram (India)	C	3.82				6.2		1.2	4.9
Meal, Bengal gram (India)	M	3.49		6.9	2.3	6.4		0.6	5.0
Meal, Chick-pea (Mexico)	M			8.2	2.1	5.7		1.5	5.0
Meal, Gram (India)	M	4.19		4.3	3.4	9.2		0.8	4.6
Galega (Galega officinalis, Goat's Rue*)*									
Seed protein	PX	12.77		5.4	5.7	5.6	5		8
Thallus protein	PX	10.85		5.5	2.4	7.9	2		3

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.7							19.5	4.6	0.4				Barré, '53
.8	0.7	4.0					21.0	7.2	3.0				Barré, '53
.7	0.6	4.2					20.8	7.4	2.6				Barré, '53
	4.9		2.8	7.2	3.7	4.8							Horn, '50
	0.5		3.3	8.7	4.6	5.3							Massieu, '50
.2	0.6		2.6	5.0	3.2	3.5	20.2	6.1	4.1	2.1	2.6		Rice, '50
	1.6			7.3	5.3	5.3	22.0		4.8				Dunn, '50d
	1.3		3.1	6.1	5.3	5.2							Williams, H.H., '55
.0	1.8			11.3		2.4			5.9	2.9			Roche, '51e
	1.5		2.9	7.9	4.7	4.9							Williams, H.H., '55
		1.5		6.0		4	16.2	3.4			5.0		*Cf.* B & B, '51
.9		3.7	5.5	6.6	4.1	5.4							Horn, '50
		3.0	2.4	13.6		5.6	23.9	12.6	6.3	4.7			Underwood, '52b
.5													Yaichnikov, '54
	1.3		3.7	5.9	3.3	4.5							Horn, '49 & '50
	1.5		3.7	6.1	3.5	5.0							Horn, '52
	1.5		3.4	5.8	4.0	4.8							Kuiken, '52
.5	1.7	2.9	3.4	4.5	3.2	4.1	13.5	10.0	3.4	3.6			Block, unpubl.
.9	0.7	4.4	3.4	5.8	3.2	5.0	20.0	9.9	4.4	4.4	3.6	0	Adriaens, '54
.2	1.5												Taranova, '51
	1.0		2.9	6.1	4.3	4.6							Williams, H.H., '55
2.0	1.8	4.5	3.0	5.7	3.8	4.5	17.7		5.3				*Cf.* B & B, '51
	1.3		3.6	6.5	4.3	5.6							Horn, '50
1.1	2.3	2.7	2.7	8.0	2.2	6.1			1.0	4.0	2.0		*Cf.* B & B, '51
.4	0.7	9.2	2.7	6.2	5.6	6.2	21.0	7.0	9.2		5.7		Fang, '53
.4	0.9	10.1	2.6	6.3	6.9	6.7	20.1	7.4	9.8		5.5		Fang, '53
2.2	0.8	5.4	5.4	10.0	5.1	3.7	19.0	4.4			4.4		Fang, '50
2.5	0.8	5.6	5.6	6.1	5.0	3.4	19.4	4.2			4.2		Fang, '50
	0.5		1.3	6.1	3.7	3.6							Block, unpublished
													Cf. B & B, '51
													Vickery, '44
1.8		3.8	6.5	4.8	5.5								Horn, '49 & '50
	2.2			5.6	5.1	5.7							Miller, S., '52
			3.3				17.3	12.1					Yemm, '53
	2.0		6.8	4.3	5.4								Neidig, '52
0.7	2.5	5.0	3.4	12.1		4.7	20.0	11.4	4.4	4.8	0.9		Thompson, J.F., '52
1.1	1.9	5.4	5.4	5.9	3.7	4.6	17.2	9.6	3.3	4.1	3.7		*Cf.* B & B, '51
4.9	0.7			5.5	4.1	3.2							Deshpande, '54
													Cf. B & B, '51
0.7	1.9		3.8	6.2	6.2	4.5							Berkhout, '48-'49
	1.1		4.1	10.4	6.7	5.9							Horn, '50
0.67- 1.1	1.0– 1.4												Soifer, '52
	1.5		3.5	8.9	5.0	6.0							Baptist, '54a
0.7	0.8		2.9										Esh, '53 & '54
0.7	1.1		4.3	7.2	5.5	6.4							Vijayarhagavan, '53
	1.0		4.2	7.9	4.9	5.4							Horn, '50
	0.7		3.8	10.4	4.8	5.4							Williams, H.H., '55
	1.4		4.3	8.0	4.8	5.4							Horn, '55
	1.3		3.9	7.7	4.8	5.7							Hirsch, '52
	1.4		3.8	6.7	4.6	5.2							Hirsch, '52
0.7	1.0		3.3	7.5	4.9	6.3							Vijayarhagavan, '53
0.7	0.7		2.8										Esh, '53 & '54
0.8	1.7		4.8	8.0	6.0	5.4							Vijayarhagavan, '53
	1.0		3.2	14.8	6.2	4.5							Massieu, '50
			0.0										Lal, '50
		11	7		11		10	4	8.7	3.6	4		Kloušek, '55a
		6	10		16		5	5	6.9	7.3	4		Koloušek, '55a

	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TYR	TRY	PHE
LEGUMES									
Green Gram (Phaseolus radiatus; Mung)	M					7.3		1.3	5.1
Meal (India)	M	4.28		6.3	2.7	7.0		0.5	5.9
Meal (India)	M	4.59				6.9		1.1	6.1
Meal (India)	C	3.77				8.0		1.0	3.6
Horse Bean (Vicia fabia, Broad bean*)*									
Meal broad bean (Mexico)	M	4.22		6.0	2.0	5.5	3.1	0.9	3.4
Meal broad bean (Mexico)	M			9.4	2.5	6.3		1.2	5.2
Kidney Bean (Phaseolus vulgaris)									
Meal	M			5.9	2.4	9.2	2.8	1.1	5.3
Meal	M			6.3	2.8	7.5			5.8
Leaves (young plants)	C	(16.0)		0.6	3.6		4.0	4.7	
Leaves (older plants)	C	16.0)		0.5	5.0		4.3	3.0	
Lentils									
Meal	M			8.9	2.2	6.4			4.5
Meal (India)	M					6.7			4.9
Meal (India)	M	4.74				6.5		0.8	4.5
Meal (India)	M	4.26		7.0	2.1	5.3		0.3	4.1
Meal (India)	C	3.82						0.9	
Meal (Mexico)	M			7.6	2.0	5.3		1.0	4.2
Mexican Bean									
Meal	M			4.7	2.5	6.0		1.0	5.4
Meal, cooked	M			4.7	2.4	5.7		1.0	5.3
Meal, various	C						4.7	0.9	5.4
Lima Bean (Phaseolus limensis)									
Meal	M			6.4	3.0	7.6			5.6
Leaf meal	M			4.2	1.3	3.6		1.4	7.0
Leaf meal, protoplasts	M			5.5	1.5	3.8		1.7	7.4
Leaf meal, material soluble in formic acid	M			5.7	1.9	5.2			7.0
Lupine (Lupinus spp.*)*									
Meal	M			6.2	1.9	3.8	2.6	1.0	3.9
Meal, an average	C				2.9	3.1	5.6		4.5
Meal (White lupine, *lupinus albus*)		(16.0)		8.3	3.0	5.2	10.0	0.8	5.1
Meal (Sweet blue lupine)	M			13.3	3.1	3.8	3.1	0.8	3.3
Seed	C			7.9					
Seed	PX			11.7	5.9	6.4	7.2		7.5
Conglutin, from lupine seeds		16.5	1.1	12.2	2.0	4.1	2.8	0	3.8
Navy Bean (Phaseolus vulgaris)									
Meal	M			6.9	2.9	7.9			6.1
Meal (Pillsbury)	M			5.2	2.8	6.3			5.6
Meal, (Quaker Oats)	M			4.5	2.9	6.7			5.6
Meal (Staley)	M			4.0	2.6	5.6			5.2
Meal, hulls removed	M			5.0	3.1	7.0			5.1
Meal, with hulls	M			5.0	2.9	6.4			5.3
Pea (Pisum sativum)									
Meal	CM	(16.0)		7.6	2.4	7.7		0.8	4.3
Meal	M	0.72		10.1	1.8	7.0		0.8	4.2
Meal	M	4.15							
Meal	M	0.56		11.4	1.7	6.8		1.1	4.5
Meal, an average	M	4.50		7.7	1.7	6.0		0.8	4.6
Meal, field peas	M			7.2	3.2	6.2	4.1	1.2	5.0
Meal, green peas	M			9.7	2.3	7.5			4.8
Meal, green peas (Continental)	M			7.3	2.3	6.9			4.6
Meal, green peas (Quaker Oats)	M			6.8	2.2	6.5			4.4
Meal, dried (India)	C	3.36				7.0		1.1	3.7
Meal, dried (Mexico)	M			7.9	1.8	5.7		1.1	4.9
Meal, immature peas	M			6.8	0.9	1.4		0.7	2.5
Meal, nearly mature peas	M			7.3	1.2	3.2		0.7	3.0
Leaf Meal	M			4.6	1.6	4.9		1.5	6.0
Seed	C			6.4					
Seed	PX			10.8	4.6	8.2	5.8		5.6
Vine (material soluble in formic acid)	M			5.4	1.8	5.8			6.7
Isolated Proteins:									
Albumin	CX	15.1		5.4	3.4	10.9	5.8	2.4	
Legumelin, an average	C	18.0		4.9	2.0	2.7			4.6
Legumin	CX	17.9		11.7	2.7	3.1	3.8	1.2	
Vicilin	CX	17.5		10.5	2.2	4.2	3.8	0.4	
Vicilin	C	17.1		8.3	2.1	5.0			
Pinto Bean, meal	M			5.4	2.9	6.8		1.0	6.2
Red Gram									
Meal (India)	C	3.53				6.2		0.9	[illegible]
Meal (India)	M	4.14		5.4	3.4	6.4		0.2	[illegible]
Ryokuto Bean (Phaseolus Mungo, var. *radiatus;* Japan*)*	C	14.9		6.2	2.1	3.3	2.4	1.2	3.1
Vetch									
Meal	C	(16.0)		9.9	1.3	3.2	1.8	0.8	
Seed *(Vicia sativa)*	C			8.7					
Seed *(Vicia sativa)*	PX			12.9	5.3	5.7	7.0		[illegible]

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
	1.2		3.5	7.8	4.8	5.8							Baptist, '52
.6	1.0		3.5	7.6	6.3	6.4							Vijayarhagavan, '53
	1.2		3.5	8.3	4.8	6.0							Baptist, '54a
.4	0.9		2.8										Esh, '53 & '54
.2	0.5	5.7	2.6	7.6	5.5	5.6	13.7	6.5	3.7	2.6	4.5		Massieu, '49
	0.3		3.7	10.2	7.1	4.7							Massieu, '50
	1.0		3.5	7.7	4.9	6.0							Williams, H.H., '55
	1.1		4.6	7.6	5.0	5.8							Horn, '55
.0													Osipova, '53
.6													Osipova, '53
	0.7		3.5	6.9	4.4	4.8							Horn, '55
	0.7		3.7	6.9	4.8	5.5							Baptist, '52
	0.7		3.5	6.9	4.5	5.0							Baptist, '54a
.8	0.8		2.9	5.4	5.5	5.3							Vijayarhagavan, '53
.3	0.4												Esh, '53 & '54
	0.4		3.1	13.1	6.4	5.3							Massieu, '50
	0.7		5.0	13.2		5.3							Massieu, '49
	0.7		4.8	11.8		5.1							Massieu, '49
													Baldovinos, '55
	1.2		4.8	8.2	5.6	5.8							Horn, '55
	1.2		4.0	6.6	3.6	5.0							Kelley, '53
	1.2		4.6	8.0	4.7	5.6							Kelley, '53
	2.2		4.7	9.1	5.2	5.9							Kelley, '53
0.7	1.0	2.7	2.0	7.5	3.7	4.6	23.0	5.9	2.0	3.9	3.9		Ågren, '49a
2.4	0.6			9.0			27.2	5.4			4.0		*Cf.*, B & B, '51
1.0	1.8	2.7	7.3	10.9		3.7	14.2	6.2	2.5		2.0		Hanson, '52
0.5	0.6	6.1	3.1	0.5	4.5	5.0	24.4	12.7	5.7	3.8	5.0		Ågren, '49a
													Holmes, '51
	1.9		4.5	10.9	7.8	7.4	24.9	10.2	3.9	2.7			Holmes, '53a
4.0	0	5.0	2.3	8.8	5.9	3.6	25.5	10.5	0.8	2.5	4.0		Petri, '53
	1.2		5.2	8.0	4.8	6.4							Horn, '55
	1.0			8.0	6.4	5.8	15.0		4.0				Dunn, '49d
	1.0			7.9	6.4	5.8	15.0		5.3				Dunn, '49d
	1.0			8.0	6.0	5.6	14.0		4.3				Dunn, '49d
	0.8		5.1	8.7	6.3	6.0	15.0		4.8				Dunn, '49d
	1.0		4.9	8.4	6.2	5.9	15.0		4.8				Dunn, '49d
0.7	1.8		3.9	6.5	6.4	4.2							Berkhout, '48-'49
	0.7		4.2	7.2	4.2	4.6							Anderson, '53
					4.8								Horn, '49
	0.7		4.0	7.7	4.3	5.4							Anderson, '53
1.2	1.1		3.9	6.6	5.0	4.6							*Cf.* B & B, '51
1.5	1.5		3.8	6.4	5.3	5.3							Edwards, C.H., '55
	0.8		3.9	7.5	4.6	4.9							Horn, '55
	0.8			6.9	6.0	5.1	16.0		4.5				Dunn, '49d
	0.7			6.7	5.6	5.0	16.0						Dunn, '49d
0.5	0.3		2.8										Esh, '53 & '54
	0.3		3.9	9.8	7.0	4.9							Massieu, '50
	0.6		4.7	4.7	3.7	3.1							Chitre, '50
	0.8		3.3	7.2	4.7	3.3							Chitre, '50
	1.0		4.4	7.8	4.4	5.7							Kelley, '53
													Holmes, '51
	1.7		4.1	7.9	4.8	4.2	31.8	8.7	6.1	3.8			Holmes, '53a
	1.8		4.5	8.7	5.1	6.1							Kelley, '53
							17.3	15.4					Danielsson, '52
													Cf. B & B, '51
							26.9	14.6					Danielsson, '52
							20.8	14.4					Danielsson, '52
		4.2	3.3										*Cf.* B & B, '51
	1.0		4.3	8.2	5.7	6.2							Hirsch, '52
0.4	0.3		2.3										Esh, '53 & '54
0.8	0.9		3.4	7.0	5.7	5.1							Vijayarhagavan, '53
0.4	0.5			5.4	3.2	4.1	18.9	8.1	5.6	4.3	2.4		Sasaki, '41
													Yaichnikov, '54
1.1													Holmes, '51
	2.6		5.2	7.2	5.7	7.9	17.5	13.4	3.9	6.6			Holmes, '53a

	Meth-od	Nitro-gen	Sul-fur	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PH*
LINSEED (FLAXSEED)									
Meal *(Linum usitatissimum)*	C	(16.0)		9.4	1.5	3.2	1.3	1.1	
Meal	M			9.9	2.7	3.5	2.2	1.4	3.9
Meal	M			8.6	2.0	3.7	4.1	1.2	4.3
Meal	PX			12.1	4.2	10.0	4.3		5.3
Meal, an average	CM	6.9	1.1	7.7	1.6	3.0	5.1	1.7	5.4
Meal, flax cake	X	5.44		9.6	1.8	3.3	2.4	1.3	4.6
Meal, oil meal	M			8.6	1.9	4.1	2.2	1.5	4.1
PALM (see also COCONUT)									
Babassu kernel oil meal	M			12.6	1.7	4.2	1.9	0.8	4.2
Palm meal				11.0	1.8	6.7		1.7	
Tucum oil meal	M			9.5	1.5	4.8	2.7	0.8	3.1
PEAS, see LEGUMES									
PEANUT									
Meal				10.4	5.7	6.0		1.8	4.6
Meal	M	10.15		12.2	2.1	3.0		0.9	5.2
Meal	M								
Meal	M			10.0	2.9	3.2	3.2	1.4	4.6
Meal	M			11.3	2.2	3.5	3.0	0.8	4.9
Meal	M			11.5	2.2	3.2		1.1	5.3
Meal	M	10.16		8.6	2.1	2.5	2.8	1.5	4.3
Meal	M					3.1–3.4			
Meal (Spanish)	M			12.0	2.3	3.8			5.2
Meal	X				2.0		4.4		6.4
Meal, an average	C	10.4		11.0	2.2	2.9	4.1	1.3	5.0
Meal, protein	M			14.0	2.0	3.1			5.4
Meal, extracted	M			11.2	2.2	4.4	3.7	0.9	5.0
Meal, raw (Spanish)	M			10.6	2.8	3.9		1.1	5.6
Meal, roasted (Spanish)	M			8.7	2.8	3.9		1.0	4.6
Meal, roasted	M			10.7	2.1	2.8	4.6	0.9	5.4
Isolated proteins:									
Arachin	C	18.29			2.0				
Arachin	C	18.0		9.9	1.7	3.6	3.6	0.7	
Arachin	M	18.3		11.8	1.9	2.4		0.8	6.1
Arachin, an average	CM	18.3	0.42	12.2	1.8	3.1	4.7	0.8	5.5
Conarachin	M	18.2		14.5	1.8	4.1		1.1	3.8
Globulin	M	18.8		12.6	1.5	3.1		0.8	5.1
PECANS	M			10.4	3.0	5.4	2.7	1.4	5.3
RAPESEED *(Brassica Napus)*									
Meal	M	9.1		5.6	2.6	3.5	2.3	2.0	4.0
Meal, oil meal		(16.0)		7.2		5.4	6.6		
SESAME									
Meal	C								
Meal	M			10.0	2.4	2.7	4.7	1.2	4.5
Meal, an average	C	6.1		8.1	1.5	2.7	3.5	2.0	7.4
SOYBEAN									
Meal	M			5.7	1.5	5.3	2.6	1.0	3.8
Meal	M	8.85		7.8	2.4	7.2		1.1	4.8
Meal	M			7.3	3.0	8.2			5.5
Meal	M			8.3	2.6	6.8	3.4	1.0	5.0
Meal	M					6.7		1.3	5.1
Meal	M			7.0	2.5	6.6	4.1	1.1	5.3
Meal	M			8.2	2.6	6.8			5.0
Meal	PX	8.39		7.6	3.1	5.9	2.9	1.3	3.4
Meal (Staley Co. flour)	M			6.2	2.5	6.1			5.0
Meal, extractor process	M			6.0	2.3	6.4	3.1	1.2	4.8
Meal, expellor process	M			6.0	2.4	6.3	3.2	1.3	4.8
Meal, raw	CM								
Curd	CM								
Milk	CM								
Root nodule	X			6.0	3.5	6.4	1.6		4.4
Isolated proteins:									
Alpha protein	M			7.7	2.6	6.1	3.4	1.0	5.9
Glycinin	M	17.3		7.3	1.8	6.4		1.0	5.4
Globulin	M			8.3	2.2	6.5			6.0
Globulin A	C	17.04			2.0				
Globulin B	C	16.94			2.0				
SUNFLOWER SEED									
Meal	C	(16.0)		9.4	2.0	3.1	2.2	1.1	
Meal, an average	CM			8.2	1.7	3.8	2.6	1.3	5.0
Meal (flour)	M			7.6	2.3	3.6			4.3
Meal, low temperature process	M			9.3	2.1	3.2		1.0	4.2
Meal, commercial process	M			8.7	1.1	2.8		1.0	4.3
TALLOW NUT, Chinese	M	(16.0)		16.6	2.9	2.6	3.7		
TOBACCO SEED, globulin	M	(16.0)						1.5	5.7

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.0													Yaichnikov, '54
.9	1.5	6.3	3.9	6.3	5.6	5.9	21.6	11.8	6.7	2.8	4.8		Ågren, '51
	1.1		3.5	5.9	4.8	4.7							Williams, H.H., '55
	2.4		5.3	7.5	3.9	6.6	26.2	9.6	3.9	6.4			Holmes, '53a
.1	2.1				6.4	3.8							*Cf.* B & B, '51
.9	1.2	4.8	4.1	6.4	4.1	4.7	18.4	9.6	5.6	4.7	3.4		Dustin, '53a
	1.0		3.6	5.8	5.9	4.9							Williams, H.H., '55
	1.5		2.9	6.3	5.1	4.8							Williams, H.H., '55
.7	1.2			5.5		4.4			4.1	4.3			Baudouin, '50
	1.1		2.2	6.0	3.4	3.4							Williams,H.H.,'55
.7	0.5		1.6	2.6		6.9			4.2	8.8			Baudouin, '50
	0.8		3.0	6.7	3.8	4.5							Horn, '49 & '50
.9	0.8	6.6	2.6	6.6	4.6	5.7	21.7	15.1	5.7	2.9	5.2		Agren, '51
.4	0.8		2.8	7.1	4.1	4.6	18.3	13.0	5.4		5.0		Rutgers Univ., '50
	0.8		2.8	6.1	4.6	5.3							Miller, S., '52
.7	0.6		2.3	5.7	3.4	3.7							Frost, '49
													Carpenter, '51
	1.1		2.8	6.3	4.4	4.4							Horn, '55
	0.8			6.8	3.4	4.6							Miller, S., '52
1.6	0.9		2.4	6.5	4.0	4.4	19.3	14.0	5.6	4.2			*Cf.* B & B, '51
	1.1		3.4	6.7	4.0	4.7	20.0	12.0	4.1				Murphy, '50
	0.7		2.8	7.0	3.8	5.4							Williams, H.H., '55
	0.9		2.9	5.0	4.5	5.5							Hirsch, '52
	0.9		2.6	4.7	4.0	4.9							Hirsch, '52
1.0	1.0		2.8	6.5	3.5	4.6							Edwards, C.H., '55
													Vickery, '44
1.0	0.5	4.1					15.1	4.4	1.6				Barré ,'53
	0.2		2.4	6.6	4.0	4.2							Horn, '49 & '50
1.0	0.8	4.6	2.2	5.8	6.6	3.9	17.4		3.2		6.0		*Cf.* B & B, '51
	1.9		1.7	5.8	3.5	3.3							Horn, '49 & '50
	0.9		2.9	6.6	3.8	4.4							Horn, '50
2.1	1.7		4.0	8.2	4.9	3.5							Edwards, C.H., '55
1.7	1.1	8.6	3.8	5.7	3.7	5.7	17.1	9.7	6.3	1.9	8.0		Ågren, '52d
1.9	5.3		4.8	8.7	4.5	6.5				5.0			Bell, '55
2.7	3.4												Chang, '49
	2.0		3.5	6.1	3.6	4.8							Williams, H.H., '55
1.3	3.3		3.1	8.0	4.8	5.1			9.3	3.5			*Cf.* B & B, '51
0.7	1.1	5.2	2.6	7.2	4.3	4.8	16.0	6.7	1.7	5.7	4.3		Ågren, '49a
	1.0		4.6	7.5	4.9	4.9							Horn, '49 & '50
	1.3			8.6	7.4	5.9	20.0		4.4				Dunn, '49d
0.6	1.0	6.9	3.9	7.5	6.5	5.5	19.5	6.2	4.1	3.6	2.5		Drackett Tech. Bull.
	1.4		3.8	7.7	5.1	5.4							Baptist, '54a
1.2	1.2		4.0	7.9	6.3	6.7	20.5	8.8	4.2		4.8		McClure, '53a
	1.3		4.2	7.5	5.5	4.7							Horn, '55
1.7	1.0	4.7	4.2	7.6	4.3	4.5	19.3	11.4	3.8	4.3	4.7		Block, Unpublished
	1.2			7.7	6.4	5.3	16.0		4.6				Dunn, '49d
	0.7		3.8	6.6	6.4	5.0							Williams, H.H., '55
	0.7		3.8	7.8	6.4	4.9							Williams, H.H., '55
1.8	0.78-1.1												Chang, '49
1.2	0.8-1.2												Chang, '49
2.3	0.9-1.4												Chang, '49
0.8	1.6	4.4	5.1	7.8	4.1	7.0	11.5	11.3	3.9	7.3	4.3		Zelitch, '52
	1.3		3.7	8.1	5.6	6.6							Williams, H.H., '55
	1.1		2.8	7.5	5.3	4.2							Horn, '50
	1.2			8.5	7.1	5.4	23.0		4.0				Dunn, '49
													Vickery, '44
													Vickery, '44
1.8													Yaichnikov, '54
1.4	2.9		3.9	6.8	5.2	5.4							*Cf.* B & B, '51
	1.6			6.7	6.2	5.1	18.0		5.0				Dunn, '50d
	1.6		3.2	6.0	4.0	4.9							Renner, '53
	1.5		3.2	5.9	3.9	4.8							Renner, '53
1.3	1.6			7.4		7.8	17.3	11.7	4.9				*Cf.* B & B, '51
				10.5	6.1	6.7							*Cf.* B & B, '51

Description	*Meth-od*	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PH*
BLUE-GREEN									
Anabaena cylindrica	PX			5.8*	1.5*	5.6*	3.3*	1.2	5.5
Colotrix parientina	X	3.71					1.1		2.0
Diplocystis aeruginosa	X	4.13							4.4
Nostoc muscorum	M	7.0		11.3	1.8	5.3		0.5	8.9
Nostoc muscorum	X	6.05					0.8		4.9
FRESH-WATER									
Chlorella	M			6.0	1.6	6.1		1.0	5.4
Chlorella pyrenoidosa	M			6.1	2.0	5.1	1.8	1.2	5.0
Chlorella pyrenoidosa	M	7.4		5.6	0.6	4.7	1.7		11.0
Chlorella vulgaris	M			3.8	1.4		3.2	1.1	11.6
Chlorella vulgaris	PX	4.3		15.8	3.3	10.2	2.8	2.1	2.8
Chlorella vulgaris	PX	6.1		7.9	1.9	8.6	5.8	2.5	5.3
Chlorella vulgaris	X			7.0 8.8	2.1 3.5	7.0– 7.7	5.4– 5.8	2.1– 2.5	4.1 4.3
Tribonema aequale	PX			7.9	2.2	7.6	6.3	2.2	5.3
MARINE									
Brown							1.6 5.7	0.9 9.8	
Fucus vesiculosis	X			4.7	0.9	5.1	2.5		4.9
Green							1.0– 1.7	0.4 2.5	
Navicula pelliculosa	PX			4.6*	1.7*	7.0*	3.9*	1.3	6.4
Red							1.5– 4.8	0.5 2.4	

* Estimates (probably low).

per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
tr.	2.0	2.9	7.7	9.3	5.9	9.4	9.4	11.6	4.7	6.1	6.6		Fowden,'54
	1.2	4.0	3.4			4.1	8.4	5.8	4.5	6.1	3.0		Williams, A.E., '52
	1.8	3.3	3.2	4.2	2.2	4.1	6.5	4.6	4.9	5.4	3.2		Williams, A.E., '52
	0.9		4.7	6.9	3.7	3.7							Lazaroff, '52
	0.4	2.6	4.5	4.2	2.8	3.7	6.0	7.9	5.1	8.4	2.9		Williams, A.E., '52
	1.4		4.8	5.0	4.2	6.7			5.5				Combs, '52
.8	0.5		4.3	8.7	5.3	5.7	8.7	7.9	4.3	5.6	4.0		Schieler, '53b
.9	0.4		1.1	8.2	5.0	5.4	8.2	7.3	4.1	5.2	3.7		Schieler, '53a
.6	1.0		2.3	8.4	5.9	5.3	7.6	6.3	4.8	5.2	3.5		McClure, '53a
.2	1.4	3.3	2.9	6.1	3.5	5.5	7.8	6.4	6.2	7.7	7.2		Fowden, '52b
.3	2.4	4.0	3.9	9.2	5.1	7.4	13.1	10.7	5.3	7.8	7.6		Fowden, '54
.2–	2.0–	3.5–	3.7–	8.4–	4.6–	7.0–	11.6–	7.9–	4.9–	8.0–	5.9–		Fowden, '52a
.3	2.4	4.3	4.8	9.2	5.2	7.9	13.3	9.9	5.4	9.4	6.2		
tr.	2.4	2.9	5.4	9.6	6.2	10.0	7.7	8.6	5.3	8.5	8.0		Fowden, '54
		4.2– 20.4	1.5– 4.7										Takagi, '55
	0.7	4.2	4.5	7.5	4.5	4.0	18.8	13.7	4.6	5.5	4.3		Smith, D.G., '53
		5.0– 14.3	0.9– 2.7										Takagi, '55
tr.	2.0	5.0	5.7	10.8	5.1	10.0	8.2	10.7	5.2	6.6	8.2		Fowden, '54
		6.9– 19.6	1.1– 5.6										Takagi, '55

	Meth-od	*Nitro-gen*	*Sul-fur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PHE*	*CY*
BACTERIA										
Aerobactor aerogenes, protein from	M	(16.0)		8.5	5.7	4.9	7.2		4.8	1.2
Bacillus brevis	X	10.9		1.3	1.6	1.7	+		16.3	?
Chlorobacterium sp.	X			6.2	1.7	9.5	2.6		6.4	2.3
Chromatium sp.	X			6.1	3.2	5.2	4.1		5.1	1.2
Escherichia coli (Culture 1)	M	(16.0)		6.5	0.5	3.5	0.7	0.7	1.2	2.2
Escherichia coli (Culture 2)	M	(16.0)		5.5	0.6	6.0	2.4	0.5	1.8	2.4
Escherichia coli (Culture 3)	M	(16.0)		4.5	0.8	3.7	1.6	0.5	1.8	1.8
Gaffkya tetragena	M	(16.0)						0.4		
Lactobacillus arabinosus	M									
Leuconostoc mesenteroides	M									
Mycobacterium tuberculosis	M	10.6		7.4	0.5	6.2	1.8	2.5	5.8	2.2
Mycobacterium tuberculosis, H37RV	X	Rel. M.R.		9.3	2.4	3.7	1.5		2.6	
Mycobacterium tuberculosis, H37RV	X	Rel. M.R.		9.0–	2.0	3.4	0.7		1.3	
				9.5	2.8	3.9	1.8		3.0	
Mycobacterium tuberculosis, H37RVSR	X	Rel. M.R.		9.4	2.5	4.1	1.5		2.4	
Mycobacterium tuberculosis, H37RVSR	X	Rel. M.R.		8.0–	2.0	3.0				
				10.4	2.9	5.6				
Nitrosomonas sp.	PX			1.0	0.6	3.4	0.2		7.5	
Pseudomonas aeruginosa	M	(16.0)		4.6	0.9	6.3	0.9	0.6	3.2	3.8
Rhodospirillum rubrum	X			8.0	1.5	4.5	1.8		5.1	2.1
Salmonella enteritidis	M	(16.0)		4.5	0.9	4.5	1.5	0.4	2.8	1.2
Salmonella paratyphi A	M	(16.0)		7.5	0.8	4.5		0.5	2.7	1.2
Salmonella Schottmüller i	M	(16.0)		4.0	0.9	7.2	1.8	0.5	3.3	2.4
Salmonella typhosa (Eberthella typhosa)	M	(16.0)		6.8	0.9	7.2	1.5	0.5	2.7	1.9
Shigella dysenteriae	M	(16.0)		7.5	1.0	4.1	2.0	0.5	2.6	1.8
Staphylococcus aureus	M	(16.0)		2.6	0.9	6.5	2.4		2.0	0.8
Staphylococcus aureus	M	(16.0)		3.7	0.7	3.4	0.8	0.5	3.0	1.8
Streptococcus faecalis	M									
Streptococcus hemolyticus	M	(16.0)		6.0	1.2	6.0	0.8	0.5	2.6	2.0
Streptococcus hemolyticus	M	(16.0)						0.4		
Various bacteria, an average	M	10.4		3.9	1.9	5.9		1.7	3.3	2.2
BACTERIAL SUBSTANCES										
Fecanin (Human)	C			6.4	3.3	13.7				
Feces (Ruminant)	M			3.4	1.8	5.1		0.9	3.4	
Rumen bacteria (Dry-fed)	PX			8.1	2.8	5.2	5.2	1.6	4.1	
Rumen bacteria (Green-fed)	PX			5.9	3.7	4.2	3.9	1.4	3.8	
Rumen contents (0 hours)	M			5.0	2.2	5.7		0.6	3.4	
Rumen contents (6 hrs. after urea ingestion)	M			4.8	2.3	6.5		0.6	3.8	
Rumen contents	M			3.1	1.4	5.7		0.6	2.5	
Tuberculin	X	M.R.			0.3	5.6	1.6		2.1	1.2

acteria and Bacterial Substances

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

ET	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*Other AA's*	*Reference*
3.0	5.3	5.3	8.5	6.0	5.9	13.9	7.8	6.3		4.3		Sussman, '54
+	2.3	3.1	+	+	+	+	13.9	2.1	+	7.6		Stevens, C.M., '55
2.1	4.0	4.0	6.3	4.9	7.6	12.8	7.1	5.7	6.9	5.5		Wall, '52
2.5	2.8	4.4	7.1	5.7	7.0	15.2	6.3	5.7	7.8	6.1	β-Alanine, 0.6	Wall, '52
1.0	1.2	4.5	6.5	5.5	5.5	13.0	15.0	1.2	2.6	0.6		Mondolfo, '51a
0.6	1.2	4.0	5.0	5.5	6.0	12.5	9.0	1.2	4.5	0.4		Mondolfo, '51a
0.4	0.7	2.7	4.5	3.7	3.0	15.0	12.0	1.2	2.0	0.4		Mondolfo, '51a
0.5		2.8	5.5	5.5	3.6	13.0						Mondolfo, '51a
							9.1–9.3				L-Aspartic acid, 8.5-9.2	Camien, '51
							10.8–11.3				L-Aspartic acid, 7.6-7.9	Camien, '51
1.2	4.5	4.9	10.6		6.4	7.3	1.5	3.5	12.3	3.5	γ-Aminobutyric acid, +	Eguchi, '51
2.5	4.8	5.4	8.3	4.2	6.2	9.0	6.9	7.8	12.6	2.6		Nishihara, '53
1.0	4.0–	4.7–	6.5	3.3–	5.0–	9.3–	6.1–	6.1–	11.5–	1.9–		Nishihara, '53
3.8	6.8	6.9	10.7	7.3	7.1	10.9	8.3	7.8	15.1	3.2		
2.7	5.0	5.5	9.0	3.8	5.6	10.5	6.9	7.7	12.4	2.6		Nishihara, '53
												Nishihara, '53
3.4	2.4	5.4	4.5	6.0	9.4	13.2	7.6	6.8	8.1	5.3		Hofman, '53ab
0.5	1.5	2.2	4.5	3.7	3.6	15.7	24.0	1.5	3.0	0.8		Mondolfo, '51a
2.5	5.0	5.1	7.6	4.2	7.3	13.5	9.3	6.1	9.1	2.8		Wall, '52
0.5	0.8	3.2	4.2	3.7	3.3	17.5	13.5	1.2	4.1	0.6		Mondolfo, '51a
0.5	0.5	3.5	5.2	4.5	4.5	14.7	16.2	1.0	2.9	0.5		Mondolfo, '51a
0.5	1.0	3.2	3.0	3.7	3.3	18.2	21.0	1.5	3.2	0.6		Mondolfo, '51a
0.5	0.9	4.3	4.5	3.0	3.3	18.5	16.2	1.2	2.4	0.5		Mondolfo, '51a
0.5	1.4	2.7	4.7	3.0	3.6	18.7	15.0	1.5	3.1	0.5		Mondolfo, '51a
0.8	1.8	3.2	4.0	4.0	3.2	14.5	22.0	1.3	3.2	0.5		Mondolfo, '51a
0.5	0.8	3.2	4.5	4.5	2.8	19.0	13.5	1.3	2.4	0.5		Mondolfo, '51a
							9.1–9.3				L-Aspartic acid, 6.8-7.3	Camien, '51
0.4	1.0	3.8	4.5	2.8	3.0	15.0	18.6	1.5	2.4	0.7		Mondolfo, '51a
0.6		4.5	4.0	4.8	4.0	15.0						Mondolfo, '51a
1.6		3.8	6.5	5.3	5.3	9.3						B & B, '51
		8.0		6.6								B & B, '51
1.5		4.8	4.4	3.7	4.9							Loosli, '49
1.9		3.9	6.7	4.2	5.0	19.3	10.9	2.5	3.0			Holmes, '53b
2.0		3.5	6.0	4.0	6.6	20.3	10.6	3.4	4.1			Holmes, '53b
1.4		4.2	6.3	3.4	5.8							Duncan, '53
1.5		5.1	6.8	4.2	5.7							Duncan, '53
1.6		4.0	5.0	3.4	3.8							Loosli, '49
	6.0	5.7	10.9				6.1	9.3		2.5		Nishihara, '53

	Meth-od	*Nitrogen*	*Sulfur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PH*
Ustilago maydis	C	2.98		5.8	12.8	4.0			
Funaria hygrometrica (plant juice protein)	X			6.6		7.1	7.9		9.6
Mold, an average value	CM	6.8		3.9	3.5	3.9	1.3	2.0	1.9
Mucor mucedo (plant juice protein)	X			6.1	0.9	3.2	4.3		1.9
Mycelium, penicillin	M			4.0	1.3	4.0	2.7	1.0	2.7

* includes glutamine.
† includes asparagine.

Brewer's yeast	M			2.6	2.6	5.9	2.9	1.7	4.4
Brewer's yeast	M	7.71		4.7	1.6	7.2		0.9	3.7
Brewer's yeast	M			3.7	1.9	6.6			4.0
Brewer's yeast	M			5.0	2.0	7.0	2.7	1.4	3.8
Sulfite Liquor, Food Yeast from	M	7.6		7.6	2.8	8.7		1.4	5.1
Sulfite Yeast	M			4.4	2.4	4.9	2.8	0.9	4.0
Torula candida arborea	C	7.41	2.1	3.0	0.9	3.3	3.6	1.6	5.3
Torula utilis	C	6.99	0.41	2.6	0.9	2.7	2.9	1.8	3.9
Yeast Autolysate	M	12.2		4.3	2.1	7.4	2.3	1.3	3.5
Yeasts, Average values									
20 yeasts (as % dry weight)									
17 yeasts	M			4.0	2.0	6.9	7.7	1.5	3.9
12 Brewer's yeasts	PX	7.8		5.4	2.5	6.6	3.3	1.1	3.0
4 Primary food yeasts	PX	8.3		4.5	2.7	6.3	3.4	1.0	3.2
2 Torula yeasts	PX	8.7		4.0	2.6	6.3	3.3	1.0	3.2
Yeasts, composite of 6, isolated proteins	PX	8.9		5.7	3.0	10.4	4.1	2.6	4.4

COMMENTS—Microscopic Plants

Algae: The photosynthetically active chromotropin (phycocyanin of blue-green alga (Oscillatoria) shows 16 amino acids on a two-dimensional paper chromatogram of which are unknown spots and from which arginine is reported to be missing (Wassink, 1953).

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

Fungi

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
											8.7		Bachstez, '51
.1	1.5	4.1	6.0	17.0	3.2	9.6	31.6*	11.7†	5.6	6.9	5.5		Mansford, '54
	0.7		3.4	5.0	2.8	3.9							B & B, '51
.0	1.9	4.0	3.0	7.8	1.1	5.9	59.0*	24.9†	3.3	6.8	5.5		Mansford, '54
	1.5		4.2	5.6	3.6	5.6							Williams, H.H., '55

Yeasts

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.7	1.6	6.4	3.1	7.0	5.5	6.4	11.0	11.0	5.5	7.9	5.1		Ågren, '51
	1.1		5.3	6.7	4.9	5.2							Horn, '49 & '50
	1.2			7.0	6.4	5.4	17		3.9				Dunn, '49d
	1.4		4.7	7.0	6.1	5.8							Williams, H.H., '55
.4	1.8		5.4	7.5	7.9	6.3	14.5		0.4				Inskeep, '51
.2	1.1	7.4	6.4	7.4	3.9	7.4	11.3	12.8	5.4	8.2	4.9		Ågren, '51
.8	1.3		9.2				11.5	2.6		14.4	4.1	4.9	Felix, '48
.6	1.3		4.4	3.2		8.0	9.6	2.7		13.2	3.6	5.9	Felix, '48
.5	1.3	5.3	2.9	6.9	4.8	5.2	10.7	13.7	5.0	9.2	3.4		Ågren, '52d
.37	0.64												Chiao, '53
	1.3		5.1	7.0	5.9	5.9	13.6		4.2				Dunn, '45
.1	1.3	4.2	4.6	6.0	4.3	4.9	10.9	7.2	4.0	5.5	4.0	0	Block, unpublished
.0	1.4	4.2	4.9	5.3	3.9	4.7	10.9	7.2	3.9	5.5	4.0	0	Block, unpublished
.2	1.4	4.2	5.2	5.3	3.6	4.9	10.9	7.2	3.9	5.5	4.0	0	Block, unpublished
.6	2.0	7.3	7.5	8.0	6.1	8.0	7.9	10.1	4.5	7.8	4.9	0	Block, unpublished

Yeasts: A composite sample of 6 yeasts was extracted with hot acetone, ether, hot 10 per ent NaCL, hot water, acetone, ether and dried. The composition of the yeast proteins, reed of nonprotein nitrogenous compounds, is significantly different in a number of amino cids from that determined on the hydrolyzates of the entire yeast.

	Method	*Nitrogen*	*ARG*	*HIS*	*LYS*	*TYR*	*TR*
PROTAMINES							
Clupein (from *Clupea harengus*, herring)	M		44.1	0	0		
Clupein (from *Clupea harengus*, herring)	C		39.1	6.2	5.5		
Clupein (from *Clupea harengus*, herring)	CPX						
Clupein (from *Clupea harengus*, herring)	PX	as % N/TN	89.7				
Clupein (from *Clupea harengus*, herring)	PX	M.R.	53				
Fontinin (from *Salmo fontinalis*)	PX	M.R.	50				
Gallin (fromFowl)	X	24.4	34	0.8	0	3.0	0
Gallin (from Fowl)	X	24.4	38	0.9	0	3.3	
Iridin (from *Salmo irideus*, rainbow trout)	PX	M.R.	50	0			
Lacustrin (from *Salmo lacustris*, sea trout)	PX	M.R.	40–50	5–15			
Mugiline-β-Sulfate (from *Mugil japonica)*	M	uncorr.	83.2	0	0	0	0
Salmin (from *Salmo salar*, salmon)	C	uncorr.					
Salmin (from *Salmo salar*, salmon)	CM	24.5	44.5	0	0		
Salmin (from *Salmo salar*, salmon)	M	uncorr.					
Salmin (from *Salmo salar*, salmon)	PX	M.R.	55	0	0		
Salmin (from *Salmo salar*, salmon)	PX	24.5					
Salmin (from *Salmo salar*, salmon)	X		84.5				
Salmin (from *Salmo salar*, salmon)	X	M.R.	50				
Salmin (from *Salmo salar*, salmon)	X	M.R.	55	0	0		
Sturin (from *Acipenser sturia*, sturgeon)	PX	M.R.	35	7	9		
Truttin (from *Salmo trutta*)	PX	M.R.	50				
BASIC SPERM PROTEINS							
Echinoderm:							
Arbacia punctulata, sea urchin	CX		14.0	1.2	14.4	2.3	
Asterias forbesi, sea star	CX		11.8	1.7	16.0	2.3	
Echinarachnius parma, sand dollar	CX		17.0	0.6	11.7	2.3	
Loligo pealii, squid	CX		23.8	1.3	6.9	3.9	
HISTONES—*Normal*							
"Arginino-rich" (blood)	X	19.2	12.6	1.9	10.6	3.0	
Blood Erythrocyte	X	18.5	11.3	1.8	9.2	3.0	0
Blood Erythrocyte	X	18.5	12.6	2.0	10.5	3.4	
Blood Erythrocyte nucleohistone (chick)	M	16.83	10.3–10.9	1.9–2.4	13.7–15.2	2.6–3.4	0
Desoxypentose nucleohistone (liver)	X		11.3–14.0	2.4–2.5	10.9 12.6	2.9–3.7	
Desoxypentose nucleohistone (liver, rat)	X	17.2	14.8	2.7	10.7	5.0	0
Liver (calf)	X	18.0	13.1	2.0	10.3	3.5	
Liver (rat)	X		11.6	1.0	9.3	2.2	
Liver nuclei	X		11.0	2.3	10.6	3.7	
"Lysine-rich"	X	17.4	3.5	0	33.0	1.0	
Thymus, an average value	CM	16.0	10.9	2.1	13.7		
Thymus (calf)	X	17.9	10.8	2.0	8.3	2.7	
Thymus	X		15.3	2.4	9.1	2.9	0
Thymus (calf)	X		10.0	2.5	7.2	1.0	
Thymus (calf)	X	17.4	10.7	2.3	16.1	3.4	
Thymus Fraction A	X	17.95	3.4	0	31.7	1.0	
Thymus Fraction B	X	16.78	9.7	2.9	14.3	4.2	
Thymus Fraction C	X	13.99	6.1	1.2	20.9	2.8	
Thymus nucleohistone	M	16.35	11.7	2.9	12.9	4.3	0
Thymus nucleohistone, ammonia-acetone extracted	X	*	4.1	0	33.0	1.0	
Thymus nucleohistone, citric acid extracted	X	*	3.6	0	32.1	1.0	
Thymus nucleohistone, NaCl-fractionated	X	*	15.6	3.9	9.5	6.3	
HISTONES—*Pathological*							
Breast Cancer	PX		9.3	3.8	10.0	2.3	
Epithelioma, cancerous (rat)	C	18.2	22.6	3.3	9.6	1.4	
Epithelioma, cancerous, DNP-derivative, water-precipitated	PX		13.3	2.2	9.2	3.3	
Epithelioma, cancerous, DNP-derivative, $CaCl_2$-precipitated	PX		7.5	3.8	12.6	7.2	
Epithelioma, necrotic (rat)	C	17.8	22.6	2.9	10.1	1.6	
Epithelioma, necrotic, DNP-derivative, water-precipitated	PX		12.5	1.9	9.4	3.7	
Epithelioma, necrotic, DNP-derivative, $CaCl_2$-precipitated	PX		7.8	2.7	12.5	6.4	
Sarcoma (rat)	X		14.5	2.0	10.5	0.2	
NUCLEOPROTEINS							
Auxiliary Infectious N.P. from plants infected with Tobacco Mosaic Virus	PX	(16.0)	13.4		2.7	1.8	
Bakers' Yeast Ribonucleoprotein	M	M.R.	20.0–29.0	23.0–81.0	38.0–59.0	16.0–21.0	[illegible]
Brewers' Yeast Ribonucleoprotein, Fraction A	CPX	14.0	5.0	7.2	6.7	3.3	[illegible]
Brewers' Yeast Ribonucleoprotein, Fraction B	CPX	13.3	4.3	3.5	5.3	3.0	[illegible]
Brewers' Yearst Ribonucleoprotein, Fraction C	CPX	15.4	4.2	11.4	9.2	1.2	[illegible]
Clostridium perfringens (BP6K), Ribonucleoprotein from	M	(16.0)‡	8.9	2.0	15.5	5.9	0.0
Endotoxin of *Salmonella enteriditis*, nucleoprotein from	C	(16.0)	2.4	1.0	6.4	2.4	0.9
Liver, whole, desoxynucleoprotein from	X		10.7	2.5	10.6	8.0	
Liver, chromosomes, desoxynucleoprotein from	X		11.7	2.9	10.4	3.3	
Liver, nuclei, desoxyribonucleoprotein from	X		11.0	2.3	10.6	3.7	
Pancreatic nucleoprotein	PX	14.9	11.0	1.8	7.1	6.0	[illegible]

HISTONES AND NUCLEOPROTEINS

(Calculated as g. of amino acid
per 16.0 g. N *or* molar ratio)

PHE	CYS	MET	SER	THR	LEU	ISO	VAL	GLU	ASP	GLY	ALA	PRO	HYD	Reference
					0	0.5	1.8					4.1	0	*Cf*. B & B, '51
													0	*Cf*. B & B, '51
			1.7	1.0				0.0	0	1.7	0.8			*Cf*. B & B, '51
			2.4	0.6		0.4	1.6				1.9	2.2		Felix, '50
			6	2.0		1	4				4	5		Felix, '55
			3				5.0			2	2	5		Felix, '55
		0	3.9	0.8			0.8	0.7	0.4	1.8	1	2.2		Mirsky, '50-'51
		0	4.7	1.0			0.9	0.8	0.4	2.4	1.2	2.6		Daly, '51
			3			1	4			2	2	5		Felix, '55
			2			1	2	1	1.0	2	2	5		Felix, '55
0	0	1.0	2.5	2.4	0	7.1	6.1	1.4	0	0	3.5	6.5	0	Takeshita, '55
			6.7				5.0	0.2		3.2	1.2	6.7		Velick, '51b
			4.1	0	0	0.6	2.1			0	2.4	4	0	*Cf*. B & B, '51
			8.5		0	1.1	4.3	0.1				6.4		Velick, '51b
			5	0		0	4	0		3	2	5		Felix, '52 & '55
			3.6	0				0.0	0			4.4	0	*Cf*. B & B, '51
			6.3				4.7			3.2	1.1	6.9		Velick, '51b
			7			1.0	3			4	1	6		Corfield, '53
			5	0		0	4	0		3	2	5		Felix, '52
			3	1.0		2.0	0	1		2	5	0		Felix, '52 & '55
			3				5			2	2	5		Felix, '55
1.9		4.7	4.8		10.8		5.0	6.6	4.1	4.9	10.0	5.8		Hamer, '55
1.7		2.4	4.6		8.7		5.2	6.7	4.4	3.8	9.9	5.6		Hamar, '55
1.7			6.5	5.7	9.1		5.6	5.0	2.9	4.2	9.4	4.5		Hamer, '55
1.9			5.9	2.9	8.7		3.8	6.2	5.2	3.1	4.3	3.4		Hamer, '55
2.4	0.6	1.0	3.2	4.4	6.6	2.8	3.1	9.1	5.0	4.0	6.2	2.8		Daly, '55
2.7		0.0	4.5	3.9	7.5	4.8	4.5	8.5	4.6	3.8	5.9	3.0		Mirsky, '50-'51
3.0		0.0	5.5	4.6	8.7	5.5	5.3	9.7	5.1	5.0	7.4	3.5		Daly, '51
1.5– 2.0	0	0.8– 0.9	3.0 5.4	3.9– 5.9	4.8– 5.7	5.0– 6.4	3.8 4.8	7.2 9.5	4.6– 4.9	3.1– 4.6	14.7 18.1	3.7–4.4		Harper, '53
3.0– 3.2		0.7– 1.4	4.6– 5.5	5.0– 6.1	9.0– 10.2	5.1– 5.5	5.9– 6.6	10.1– 10.9	5.2– 6.4	5.7– 5.8	8.7– 9.1	3.3– 4.5		Luck, '53
2.6	0	1.5	5.0	6.0	9.4	5.3	6.0	9.8	6.5	5.4	7.5	2.5	?	Brunish, '51
3.1		0.6	3.6	5.7	8.1	4.1	4.9	9.2	4.7	5.2	6.8	3.0		Daly, '51
3.1			6.0	7.6	15.0		5.2	9.5	6.8	5.1	10.9	2.7		Eadie, '52
3.9		2.0	6.0	5.6	9.8	5.3	5.9	10.1	8.1	5.1	7.6	3.8		Luck, '51
0.9	0	0.2	4.0	3.4	4.3	1.3	3.4	3.9	2.1	3.0	10.4	5.7		Daly, '55
				5.3	6.9	5.6	4.2							*Cf*. B & B, '51
2.9	0.7	0.8	3.9	5.0	8.1	4.2	5.0	9.8	5.7	4.8	7.2	3.8		Daly, '51
3.6	0	0	4.1	4.2	4.6	10.0	6.6	3.8	5.0	4.5	6.1	3.6	0	Hamer, '50 & '51
2.2			5.6	7.2	9.1	5.5	8.2	11.6	8.1	5.3	8.7	3.9		Eadie, '52
2.6	0	1.1	4.5	5.0	7.5	4.2	5.2	8.9	4.7	4.7	8.9	4.2		Crampton, '55
0.8	0	0	6.1	5.5	5.0	1.2	5.1	4.4	2.3	4.4	18.0	8.8		Crampton, '55
1.7	0	1.0	5.6	4.5	8.1	4.4	5.7	9.1	5.4	4.6	7.7	4.0		Crampton, '55
1.7	0	0.5	5.4	5.2	6.1	2.4	4.2	6.4	3.4	4.2	13.2	6.5		Crampton, '55
2.3	0 ?	1.0	3.1	5.9	9.5	6.5	4.7	11.1	6.7	4.7	9.3		3.3	Morris, '53
0		0	5.4	6.0	3.6	1.4	5.0	2.9	3.0	5.1	21.0	8.9		Davison, '54
0		0	4.1	5 8	5.0	1.8	4.8	5.0	4.0	4.7	21.6	7.2		Davison, '54
3.7		1.9	2.2	5.9	9.4	6.6	6.3	9.7	5.1	5.1	8.0	2.1		Davison, '54
2.4	0.4		3.2	3.3	9.9		7.0	7.6	5.6	4.4	6.6	3.3		Khouvine, '54
1.3	0.3	0.3	3.8	2.8	9.1		3.6	5.4	3.3	5.5	4.8	3.3		Khouvine, '51a-'53
2.8	0.4	0.5	5.2	4.5	15.5		5.5	10.2	5.6	5.4	5.6	4.9		Khouvine, '54
2.4	0.1		1.0	2.0	18.9		10.2	6.2	10.2	5.5	9.3	2.6		Khouvine, '51a-'53
1.5	0.3	0.9	3.9	3.7	8.5		4.5	5.9	3.1	5.8	5.0	4.0		
3.2	0.4	1.7	5.2	5.6	14.2		6.7	11.1	5.2	5.6	5.7	5.8		Khouvine, '54
2.8	0.1		1.0	1.5	17.6		9.9	6.4	10.5	5.3	9.7	2.5		Khouvine, '54
4.5			4.7	6.0	4.5	15.5	6.8	2.9	5.9	5.9	4.8	3.2		Hamer, '51
7.5	0.6		6.6	9.0			6.8	12.4	9.9	2.0	5.6			Wang, '54
12.0 24.0	5.0– 7.0	5.0– 10.0	25.0– 43.0	43.0- 72.0	62.0– 152.0	35.0– 54.0	35- 46	48.0- 77.0	75.0– 111.0	56.0– 61.0	91.0– 106.0	10.0 24.0		de Robichon-Szulmajster, '53
8.1	2.6	0.5	4.2	5.4	13.8		7.2	8.2	16.0	1.0	9.8	1.6		Khouvine, '51b
4.6	0.4	0.6	8.9	9.3	23.1		7.9	9.3	16.1	4.9	7.6	2.3		Khouvine, '51b
4.5	2.5	0.6	6.0	3.2	7.0		2.5	5.9	15.0	1.1	8.3	7.9		Khouvine, '51b
6.4	1.5	1.5	5.7	5.4	9.4	10.6	8.1	14.8	17.7	3.0	5.7	0.0	0	Parsons, '53
	0.4	<3												Yagi, '49
4.7		1.5	5.8	5.4	9.6	5.6	6.7	11.3	8.2	4.8	6.8	4.9		Luck, '51
3.0		1.5	5.0	5.2	9.0	5.3	6.0	10.1	5.5	4.9	7.4	3.7		Luck, '51
3.4		2.0	6.0	5.6	9.8	5.3	5.9	10.1	8.1	5.1	7.6	3.8		Luck, '51
6.9	4.1	0 ?	4.1	0.3	4.9	0.3	7.1	13.3	14.6	1.0	4.6	5.4	0 ?	Boser, '53 & Mohnike, '54

	Meth-od	*Nitrogen*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*
Nuclei Nucleoprotein (acid protein)	C	(16.0)	7	3	7	4.5	2.5
Nuclei Nucleoprotein (residual protein)	C	(16.0)	8.5	1	3.6	1	0
Sperm nucleoproteins:							
Echinoderms, *Arbacia lixula*	M		13.9	0.9	24.1		
Echinoderms, *Brissopsis lyrifera*	M		14.2	1.1	22.5		
Echinoderms, *Echinocardium cordatum*	M		15.4	0.8	24.3		
Mollusks, *Patella coerulea*	M		42.2	0.0	6.4		
Mollusks, *Patella vulgata*	M		24.5	0.6	16.7		

* Calculated as % of total A.A. determined.
† Corrected for nucleic acid.

COMMENTS—PROTAMINES, BASIC SPERM PROTEINS, HISTONES AND NUCLEOPROTEINS

Protamines: It appears probable that the majority of the protamines for which amino acid analyses are reported are mixtures of similar polypeptides. Thus Waldschmidt-Leitz (1954) has obtained clupeins of different amino acid composition by fractionation. One fraction,

ISTONES AND NUCLEOPROTEINS

to 16.0 g. N *or* molar ratio)

HE	CYS	MET	SER	THR	LEU	ISO	VAL	GLU	ASP	GLY	ALA	PRO	HYD	Reference
	1.2	3						13	6					Zbarskii, '48
	0.3	0.6						13	6					Zbarskii, '48
			5.2	1.9	3.6	3.2	1.9			9.3	15.0	8.0		Hultin, '49
			14.0	3.8	4.5	3.6	3.8			11.4	13.2	6.6		Hultin, '49
			5.6	3.1	2.0	2.9	3.1			10.0	13.9	7.4		Hultin, '49
			6.8	1.3	2.4	0.4	2.1			3.4	2.4	2.2		Hultin, '49
			5.5	2.9	2.6	0.9	2.9			4.0	6.2	6.3		Hultin, '49

nounting to 70 per cent of the original preparation was devoid of isoleucine and contained nly one-third of the original threonine.

Nucleoproteins: Toennies (1955) says, "If the deoxyribonucleic acids are the physical orrelate of the genetic potentialities (of the cell), the implication arises that cellular differntiation may be mediated through attachment of cell-specific proteins (histones, etc.) to pecies-specific deoxyribonucleic acids."

	Meth-od	*Nitro-gen*	*Sul-fur*	*ARG*	*HIS*	*LYS*	*TYR*	*TRY*	*PH*
ALLERGENS									
Almond	M			9.2	2.5	6.3	1.7	0.4	1.
Brazil Nut	M			17.6	2.5	1.9	1.4	0	0.
Castor Bean	C			13.2	0.6	2.7	2.3	0	
Castor Bean (Brazilian variety)	M			14.9	0.8	4.1	2.3	0	0.
Castor Bean (Domestic variety)	M			13.5	0.9	4.1	2.5	0	0.
Castor Bean, Ricin	C	(19.0)		11.7	0.0	6.3	2.7	0.4	
Castor Bean, Ricin	C								
Castor Bean, Ricin	X	16.3		8.8	1.7	4.0	4.5	2.7	
Cottonseed	M			13.7	2.0	5.5	2.5	0.7	1.
Cottonseed, an average	C	17.2	1.81	15.8	tr.	2.1	3.3	0.0	
Filbert Nut (Barcelona variety	M			19.2	0.5	1.3	2.3	0.1	0.4
Filbert Nut (Du Chilly variety)	M			19.1	0.8	2.4	2.9	0.2	0.8
Filbert Nut (H-1-B variety)	M			18.4	0.8	2.5	2.7	0.2	0.8
Flaxseed	M			10.1	1.2	5.6	1.2	1.6	2.1
Kapok	M			11.0			2.9	0.4	
Mustard Seed	M			8.8	3.1	6.3	1.7	0.8	2.2
Peanut	M			5.4	3.1	10.4	3.7	0.5	1.7
Soybean	M			8.8	2.3	12.2	2.7	0.7	1.9
Walnut	M			19.3	1.8	3.3	1.5	tr.	1.6
TOXINS									
Bee Poison, Fraction I	PX	17.16		12.5	0 ?	12.0	0 ?	5.8	0 ?
Bee Poison, Fraction II	PX	15.57		13.5	4.5	14.0	2.5	2.9	2.5
Crotoxin	PX	15.86		8.6	2.3	6.4	9.7	4.2	9.7
Phalloidine (isolated from *Amanita phalloides*)	X	M.R.						1*	
Tetanal	M	15.7		3.9	1.2	10.2		0.8	5.1

* Hydroxytryptophan.

OXIC PROTEINS

per 16.0 g. N *or* molar ratio)

YS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
.1	1.9	3.1	3.3	2.1	1.5	1.6	32.3	6.1	10.8	5.0	1.8		Spies, '51b
.7	8.8	3.5	0	4.0	0.6	0.7	27.9	5.6	3.1	5.7	4.5		Spies, '51b
.8							14.5						*Cf.* B & B, '51
.9	0.9	10.9	1.1	3.6	3.2	3.8	32.9	5.8	4.2	6.7	1.2		Spies, '51b
.1	0.7	11.0	0	3.8	4.0	4.4	35.5	3.6	5.1	4.8	1.2		Spies, '51b
.0			2.7		15.0	2.0							*Cf.* B & B, '51
												< 0.1	Troll, '53a
.0	0.6								6.5				Moule, '51
.3	1.0	2.0	2.0	1.5	0.8	1.1	34.5	7.9	2.6	7.2	3.4		Spies, '51b
.3							13.6						*Cf.* B & B, '51
.9	4.1	2.0	1.8	3.9	0.9	1.2	38.8	6.2	3.0	8.5	1.7		Spies, '51b
.1	2.6	2.0	2.4	2.9	0.8	0.9	34.1	6.1	3.1	6.0	2.0		Spies, '51b
.3	2.7	2.2	2.7	3.0	0.9	1.1	34.6	6.7	2.9	6.6	1.7		Spies, '51b
.3	1.4	3.7	3.7	4.2	3.0	2.7	33.3	8.5	8.6	8.6	1.6		Spies, '51b
.5													Spies, '51b
.0	1.5	3.8	6.1	4.5	3.2	3.2	24.2	5.5	7.9	8.8	6.6		Spies, '51b
.7	1.0	7.0	5.2	2.6	2.1	2.7	13.3	7.6	25.5	5.9	2.6		Spies, '51b
.1	1.2	5.2	7.2	2.7	2.9	3.2	19.0	13.2	5.4	11.9	2.4		Spies, '51b
.9	0.9	3.1	2.0	1.8	1.5	1.1	33.0	5.2	6.2	6.5	2.6		Spies, '51b
0 ?	0 ?	3.5	7.2	17.6	9.7	5.9	8.8	1.2	5.9	5.8	3.3	0 ?	Fischer, F.G., '53a
7.8	0.8	3.4	5.6	6.3	7.3	4.3	8.5	11.5	5.1	4.8	3.4		Fischer, F.G., '53a
2.4	2.1	4.7	4.9	3.7	3.0	1.7	14.0	12.3	6.6	4.7	5.4		Fischer, F.G., '53a
1										2		2	Šorm, '53
	1.8		5.2	8.5	9.5	5.5	10.5	15.4	3.4				Dunn, '49a

	Meth-od	Nitro-gen	Sul-fur	ARG	HIS	LYS	TY
ANIMAL VIRUS, Shope Papilloma	M	(16.0)		7.0	1.8	7.0	5.8
BACTERIOPHAGE, T_3	X	uncorr.		7.2	1.7	6.3	4.2
INSECT VIRUSES							
Alfalfa Butterfly *(Colias philodice eurytheme* Bdvl.)	PX	(16.0)		19.5	1.2	2.2	6.3
Alfalfa Butterfly, Inclusion-body protein	PX	(16.0)		10.1	2.8	8.7	7.6
Caoecia murinana Hb	PX	(16.0)		16.4	1.9	3.4	5.0
Caoecia murinana Hb, Inclusion-body protein	PX	(16.0)		10.1	1.7	6.3	7.2
Eastern Tent Caterpillar *(Malacosoma americanum* F.)	PX	(16.0)		16.1	1.5	3.5	4.8
Eastern Tent Caterpillar, Inclusion-body protein	PX	(16.0)		9.7	2.8	9.4	9.6
Forest Tent Caterpillar *(Malacosoma disstria* Hbn)	PX	(16.0)		15.5	1.1	3.5	6.1
Forest Tent Caterpillar, Inclusion-body protein	PX	(16.0)		9.4	2.2	6.3	10.1
Gypsy Moth *(Porthetria dispar* L.)	PX	(16.0)		19.8	0.9	2.1	2.8
Gypsy Moth, Inclusion-body protein	PX	(16.0)		9.2	3.7	8.5	10.0
Pine Sawfly *(Neodiprion sertifer* Geoffr.), Inclusion-body protein	PX	(16.0)		10.3	3.6	6.6	5.3
Silkworm *(Bombyx mori* L.)	PX	(16.0)		11.1	0.7	3.3	6.2
Silkworm, Inclusion-body protein	PX	(16.0)		6.8	2.8	10.5	10.9
Spruce Budworm *(Choristoneura fumiferana* Clem.)	PX	(16.0)		16.3	1.6	3.1	4.7
Spruce Budworm, Inclusion-body protein	PX	(16.0)		7.9	3.2	9.8	9.4
PLANT VIRUSES							
Tobacco Mosaic Virus, an average value	C	15.9		9.3	0	1.0	4.1
Tobacco Mosaic Virus	M	(16.0)		9.8	0	1.5	3.8
Tobacco Mosaic Virus	M	(16.0)		9.7	0	1.4	3.7
Tobacco Mosaic Virus	PX	(16.0)		14.4		2.5	3.3
Tobacco Mosaic Virus Mutants:							
"B2"	M	(16.0)		9.7	0.1	1.7	3.5
"B2A"	M	(16.0)		11.0	0	2.1	4.2
"B3"	M	(16.0)		9.4	0	1.5	3.9
"B4"	M	(16.0)		10.0	0	1.5	3.8
"S1"	M	(16.0)		10.0	0	1.5	3.6
"S2"	M	(16.0)		9.6	0	1.5	3.9
"S3"	M	(16.0)		10.5	0	1.5	4.2
Tobacco Mosaic Virus Strains:							
Holmes' Masked Strain	M	(16.0)		9.9	0	1.5	3.8
TMV Mutation	M	(16.0)		10.0	0	2.0	3.9
Green Aucuba Strain	M	(16.0)		11.1	0	1.5	3.7
Yellow Aucuba Strain	M	(16.0)		11.2	0	1.5	3.7
Holmes' Rib-Grass Strain	M	(16.0)		9.9	0.7	1.5	6.8
Cucumber Virus 3	M	(16.0)		9.3	0	2.6	3.8
Cucumber Virus 4	M	(16.0)		9.3	0	2.4	3.7
Tomato Bushy Stunt Virus, a range of 3 strains	PX	(16.0)		5.7	1.2	3.1	3.1–
Turnip Yellow Mosaic Virus	M			5.9 2.5	1.3 2.1	10.7	3.4 2.5

* Cysteine.

IRUS PROTEINS

(Calculated as g. of amino acid per 16.0 g. N *or* molar ratio)

RY	*PHE*	*CYS*	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYD*	*Reference*
1.0	4.6	6.4	2.1	4.0	5.1	7.5	4.1	5.4	11.4	10.7	3.1	3.1	5.5		Knight, '50
	3.5		1.7	2.9	5.3	9.8	4.9	6.6	10.5	11.7	9.8	9.7	4.2		Fraser, '53a
	4.2	1.1	1.8	9.2	6.7	11.4		3.7	7.6	13.2	3.5	3.5	4.5		Wellington, '54
	6.4	1.3	2.3	3.6	3.0	13.2		7.0	12.8	13.3	3.0	2.8	3.37		Wellington, '54
	5.6	0.6	2.8	9.8	3.8	13.0		3.6	7.0	14.0	4.6	3.6	5.3		Wellington, '54
	9.4	1.3	3.4	3.4	6.0	14.9		5.2	12.9	11.7	2.8	2.8	5.4		Wellington, '54
	4.7	0.8	2.4	10.5	6.0	11.8		3.6	9.3	11.0	4.0	3.9	6.3		Wellington, '54
	7.0	1.3	2.4	3.6	3.1	14.3		6.9	12.4	13.5	2.7	2.9	8.2		Wellington, '54
	5.5	0.6	2.4	9.0	6.4	12.7		3.1	10.0	11.0	4.9	3.6	5.2		Wellington, '54
	7.1	1.1	2.3	4.3	4.6	13.4		5.8	11.7	13.6	2.8	2.8	6.0		Wellington, '54
	4.5	0.9	1.0	9.4	4.4	11.0		4.4	8.5	12.0	6.1	5.9	6.4		Wellington, '54
	7.5	1.1	1.7	3.8	4.7	13.0		6.0	13.2	11.1	3.1	3.3	4.9		Wellington, '54
	9.5	1.6	3.3	3.6	4.9	14.7		5.7	13.5	12.4	3.6	2.6	5.2		Wellington, '54
	4.6	0.7	2.8	8.5	9.6	11.2		3.8	6.9	13.3	8.1	5.4	4.9		Wellington, '54
	6.9	0.5	3.0	3.3	3.8	13.3		5.7	12.7	12.5	3.3	2.9	6.0		Wellington, '54
	5.4	0.6	2.4	9.2	5.0	13.7		3.9	6.9	13.2	5.0	3.5	7.6		Wellington, '54
	7.9	1.3	3.5	3.4	3.3	12.3		7.2	12.2	12.2	3.0	2.8	5.7		Wellington, '54
3.5	6.7	0.6		7.0	7.1	7.1		4.6	12.5	3.0	0	2.8	5.1		*Cf.* B & B, '51
2.1	8.4	0.7*	0	7.2	9.9	9.3	6.6	9.2	11.3	13.5	1.9	5.1	5.8		Knight, '49
1.9	8.2	0.7	0.1	9.1	11.9	8.0	5.9	10.9	11.0	11.9	2.5	7.4	5.5		Black, '53
	8.7	0.5		7.6	9.1			7.2	11.4	11.3	1.5	5.6			Wang, '54
2.0	7.5	0.8	0.1	10.6	9.9	8.0	5.2	10.4	12.1	14.2	2.8	7.7	5.4		Black, '53
2.0	8.0	0.7	0	9.8	11.1	8.1	5.7	11.7	12.7	11.2	3.3	9.6	5.7		Black, '53
2.0	7.8	0.8	0.1	10.6	13.0	8.4	6.6	12.1	11.0	12.1	2.8	9.7	5.9		Black, '53
1.9	8.3	0.7	0.1	10.9	14.3	8.0	6.6	12.7	12.5	14.6	2.5	8.5	5.6		Black, '53
1.9	6.9	0.7	0	9.5	12.7	8.5	7.3	11.6	11.4	13.5	2.3	5.9	5.3		Black, '53
1.9	6.6	0.8	0	10.5	12.9	8.6	7.0	11.6	11.6	13.5	2.3	5.6	4.8		Black, '53
1.8	6.9	0.8	0	10.8	14.1	8.7	7.2	11.3	12.0	13.0	2.5	6.6	5.2		Black, '53
2.2	8.4	0.7*	0	7.0	10.1	9.3	6.7	9.0	11.5	13.5	1.7	5.2	5.9		Knight, '49
2.2	8.4	0.6*	0	6.8	10.0	9.4	6.6	8.9	10.4	13.4	1.9	4.8	5.5		Knight, '49
2.1	8.3	0.6*	0	7.0	10.4	9.2	5.7	8.8	11.5	13.7	1.9	5.1	5.8		Knight, '49
2.1	8.4	0.6*	0	7.1	10.1	9.4	5.7	9.1	11.3	13.8	1.8	5.1	5.7		Knight, '49
1.4	5.4	0.7*	2.2	5.7	8.2	9.0	5.9	6.2	15.5	12.6	1.3	6.4	5.5		Knight, '49
0.5	9.9	0*	0	9.3	6.9	9.3	5.4	8.8	6.4		1.2				Knight, '49
0.5	9.8	0*	0	9.4	7.0	9.4	4.6	8.9	6.5	13.1	1.5	6.1	5.7		Knight, '49
0.6	3.7–3.9	0.6	0.7–1.0	5.3–5.6	8.0–8.2	8.9–9.1	2.7–3.0	7.0–7.3	4.9–5.1	9.2–9.5	4.0–4.2	4.7–4.9	2.7–2.8		de Fremery, '55
1.1	3.8	2.5	2.9	8.1	15.1	10.3	10.8	8.4	9.6	5.1	2.9	5.7	10.7	0.0	Roberts, E., '52a

	Source	Method	Nitrogen	ARG	HIS	LYS	TYR	TRY	PH
BACTERIA									
Mycobacterium phlei			11.4	7.4	1.7	3.5	2.5	0.4	3.
Mycobacterium phlei		M		7.1	1.7	3.7	2.1		3.
Mycobacterium ranae		M		7.4	1.9	4.4	2.1		2.
Mycobacterium smegmatis		M		7.7	1.9	4.4	2.5		3.1
Mycobacterium tuberculosis	Bird			7.8	2.0	3.1	2.4		3.
Mycobacterium tuberculosis	Bird		11.6	7.6	2.0	4.0	2.2	0.3	3.7
Mycobacterium tuberculosis	Bovine	M		7.7	1.9	3.6	2.0		2.
Mycobacterium tuberculosis	Human	M		8.1	2.0	3.8	2.3		3.
Mycobacterium tuberculosis	H37RU Human	M		6.4	1.7	2.9	2.2		2.
BACILLOMYCIN		X	12.29				16.6		
BARLEY									
Albumin		M		6.5	2.5	6.7		1.5	5.1
Globulin		M		11	1.8	5.3		0.8	2.8
Hordein		M		3	1.3	0.7		0.8	3.0
Hordenin		M		6	2.5	4.0		1.3	3.6
BLOOD PLATELETS			(16.0)	13		5	1.5		5
CROTOXIN				8.5	2.2	8.6	10.8	3.9	6.6
CROTOXIN, Insol. DNP fraction				10.5	2.3	8.5	(8.5)	2.6	6.6
CROTOXIN, Sol. DNP fraction				4.6	(1.4)	3.3	(4.3)	2.2	5.2
HEMOGLOBIN	Coelecanth		(16.0)	5.1	8.7		4.5	2.7	
ICHTHULOKERATIN (Egg membrane)	Salmon Egg	X		5.2	0.9	3.1	5.4	3.5	5.7
HORMONES									
Hypertensin, kidney	Rabbit	PX	M.R.	1	2		1		1
Hypertensin			M.R.	1	2		1		1
Hypertensin				1	2		1		1
Growth hormone, pituitary			15.8	10.1	2.4	7.5	4.5	1.4	9.
Melanocyte-stimulating, pituitary	Hog	PX		4.0	0.6	5.3	4.1		4.7
RICE, bran		M		10.5	3.5	6.5	5.8	4.2	5.0
RICE, polish				8.3	3.9	6.2	7.0	3.4	4.8
SCALES, fish, gelatin from	Pilchard	X	(16.0)						
SERUM PROTEINS									
Albumin	Human	PX	(16.0)	6.2	3.5			0.2	7.8
Fibrinogen	Human	PX	(16.0)	7.8	2.6			3.3	4.6
γ-Globulin	Human	PX	(16.0)	4.8	2.5			2.9	4.6
Lipoprotein	Human	PX	(16.0)	3.5	1			7.4	7.4
WHEAT GLUTEN									
65% Extraction Flour		X		3.5	1.9	1.9	3.4		5.8
75% Extraction Flour		X		3.5	2.1	2.1	3.2		5.3
100% Extraction Flour		X		4.2	2.2	2.6	2.9		4.8
WHEAT, TURKISH		M	(16.0)	6.8	2.5	2.6		0.9	4.5
YEASTS									
Brewers		M		5.4	1.8	8.2	3.3	1.1	3.8
Candida krusei		M		4.4	1.9	7.9	3.9	1.1	4.3
Hansenula anomala		M		4.4	1.7	8.4	4.4	1.2	4.7
Pichia membranae-faciens		M		4.2	1.8	7.6	3.7	1.1	4.3
Saccharomyces carlsbergensis		M		4.4	2.1	8.2	3.7	1.1	4.0

(Calculated as g. of amino acid
to 16.0 g. N *or* molar ratio)

CYS	*MET*	*SER*	*THR*	*LEU*	*ISO*	*VAL*	*GLU*	*ASP*	*GLY*	*ALA*	*PRO*	*HYL*	*Reference*
0.3	1.5		5.0	7.7	4.6	7.0	10.6	4.9			4.8		Boniece, '50
	1.6	3.1		8.1	4.3	5.9	12.0	6.4		9.8			Ginsburg, '56
	1.5	3.1		8.3	4.3	6.4	9.8	6.7		8.9			Ginsburg, '56
	1.5	3.4		8.7	4.3	6.7	10.1	6.9		9.6			Ginsburg, '56
	1.6	7.2		9.5	4.5	6.8	9.7	7.3		10.7			Ginsburg, '56
0.2	1.4		5.0	7.4	4.5	6.8	10.3	4.7			4.8		Boniece, '50
	1.6	3.3		8.8	4.5	7.0	11.6	6.5		10.3			Ginsburg, '56
	1.7	3.5		9.0	4.4	6.7	10.6	6.8		9.6			Ginsburg, '56
	1.5	3.3		8.8	4.1	6.3	16.2	6.2		11.7			Ginsburg, '56
		17.9	5.2				13.6	24.8					Turner, '56
2.1	2.4	4.9	4.6	8.6	6.2	7.8	12.9	12.2	5.7	7.3	5.5		Folkes, '56
3.6	1.5	4.7	3.3	6.8	3.3	5.5	11.9	8.5	9.2	0.7	3.6		Folkes, '56
2.1	1.3	3.8	2.6	6.9	5.4	4.7	39.6	1.8	1.5	2.2	20.1		Folkes, '56
1.2	1.9	5.0	4.2	8.7	5.2	6.6	19.8	7.1	4.5	6.7	8.7		Folkes, '56
1.5	1.5	5	5	0		5	15	10	5	5	10		Bestetti, '55
12.9	1.5	4.6	5.3		8.7	2.4	12.8	11.7	7.2	4.3	4.8		Fraenkel-Conrat, '56
(10.4)	(1.1)	4.2	5.2		9.6	2.4	10.9	9.8	6.1	4.2	3.8		Fraenkel-Conrat, '56
(10.7)	(0.9)	4.9	4.8		6.6	1.9	20.2	16.8	7.5	5.4	5.0		Fraenkel-Conrat, '56
													de Prailauné, '55
2.7	1.7	4.0	6.0	7.4	4.8	5.0	14.4	9.1	2.8	4.9	11.8		Young, '56
				1		2		1			1		Peart, '56a
				1		2		1			1		Peart, '56b
				1	1	1		1			1		Peart, '56b
2.1	2.3	5.1	6.9	22.3		3.6	16.4	10.4	3.3	6.2	3.6		Li, '56
5.3		3.4	1.3	1.7		2.9	5.3	3.8	2.3	1.4	3.7		Lerner, '55
1.3	3.9	8.2	4.3	8.1	5.6	7.0	8.2	3.6	9.2		7.0		Kik, '56
1.5	4.3	8.4	3.9	6.8	5.5	6.3	7.7	5.0	8.8		6.6		Kik, '56
	0.6	1.4	1.6	2.3	1.3	2.2	10.5	7.7	25.8	12.6	12.9	8.4	Solomons, '55
6.3	1.3	3.3	4.6	11.0	1.7	7.7	17.0	9.0	1.6		5.1		Shore, '54
2.3	2.6	7.0	6.1	7.1	4.8	4.1	14.5	13.1	5.6		5.7		Shore, '54
2.4	1.1	11.4	8.4	9.3	2.7	9.7	11.8	8.8	4.2		8.1		Shore, '54
5	0	5.4	5.9	17.3	0	5.8	10.8	10.1	2.5	4.4	3.3		Shore, '54
2.2	1.5	4.7	2.8	6.8	3.5	3.9	33.5	4.2	3.4	2.8	11.9		Nunnikhoven, '55
2.0	1.5	4.3	2.8	6.7	3.5	3.5	32.8	4.2	3.4	2.7	11.4		Nunnikhoven, '55
2.1	1.7	4.6	3.0	6.5	3.4	3.8	28.2	4.8	3.9	3.4	10.4		Nunnikhoven, '55
	1.4		3.0	6.7	4.3	4.5	27.6	4.6	3.1		11.4		Kösker, '55
0.1	1.1	2.4	3.8	6.8	3.8	5.3	11.6	12.3	4.8	5.0	3.8		Mojonnier, '55
	1.8	2.6	4.9	7.5	4.7	5.8	10.8	12.8	4.5	4.0	3.6		Mojonnier, '55
0.1	0.9	2.8	4.9	8.6	5.8	6.5	13.9	12.7	5.5	4.3	4.3		Mojonnier, '55
	1.7	2.3	4.9	8.0	5.5	6.0	13.0	12.0	4.7	4.1	3.6		Mojonnier, '55
	1.2	2.9	4.6	7.2	4.4	5.5	14.4	12.6	4.6	4.2	3.7		Mojonnier, '55

Summary Tables

The values given in the following summary tables are, wherever possible, the numerical averages of the figures given in Part II of this monograph. Inspection of the detailed tables will show that in most instances there are rather wide discrepancies in the amino acid values reported for the great majority of proteins which have been analyzed by different investigators. Therefore, *the figures in the summary tables are indicative of the amino acid composition* and should not be deemed as being absolute.

It cannot be stressed too often that, with few exceptions, the methods for the estimation of the amino acids in protein hydrolysates are not ideal; there are further errors of varying magnitude during the liberation of the amino acids by hydrolysis, and finally in the case of the nutritionally important food proteins, especially those of plant origin, large specie differences may exist. In fact just as the vitamin and mineral contents of a plant can be varied by breeding and cultivation, it is probable that the amino acid composition of the proteins of that plant can likewise vary. There may come a day when plants will be bred for their content of the essential amino acids as well as certain of the vitamins.

All the data in previous Chapters have been calculated as gm. of amino acid per 16 grams of nitrogen, the values given in the summary tables are likewise so calculated. If, for example, one wishes to know the approximate amino acid composition of a purified laboratory sample of fibrin which analyzes for 17.7% of nitrogen on a moisture and ash-free basis, then it is only necessary to multiply the proper values in Table I by the factor

$$\frac{17.7}{16.0} \text{ or } 1.11$$

If on the other hand, one wishes to know approximately how much of each of the essential amino acids is being ingested by a group of rats eating a diet which contains commercial fibrin, N = 13.0%; then these values in Table I are multiplied by the factor

$$\frac{13.0}{16.0} \quad \text{or } 0.81$$

If a sample of flour contains 12% protein (N × 5.7), then the approximate quantity of the amino acids supplied by 100 lbs. of that flour could be ascertained by multiplying the values in the proper table by the factor

$$\frac{2.1}{16.0} \quad \text{or } 0.131$$

TABLE I

BLOOD PROTEINS

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Whole Blood*	*Fibrin*	*Serum Proteins Normal*	*Serum Albumins Normal*	*Serum γ-Globulin*	*Globins and Hemoglobins Normal*	*Bence-Jones Proteins*
Arg	4.5	7.0	5.0	5.9	4.2	3.3	4.9
His	6.4	2.2	2.5	3.4	2.5	7.6	1.4
Lys	9.2	8.2	8.0	11.3	7.6	9.3	5.7
Tyr	2.5	3.9	6.1	4.9	5.9	2.9	6.1
Try	1.4	2.4	1.5	0.6	2.9	1.5	2.4
Phe	7.7	4.0	5.4	6.9	5.1	7.6	3.7
Cys	1.4	2.3	3.9	6.0	2.6	1.0	2.7
Met	1.2	2.3	1.7	1.3	1.1	1.5	0.2
Ser	8.4	12.8	3.4	4.5	10.4	5.3	10.2
Thr	4.4	7.3	5.3	5.7	7.4	5.9	8.6
Leu	11.6	8.9	13.8	11.5	9.2	14.5	7.3
Iso	2.3	4.3	2.9	2.4	2.6	0.8	3.4
Val	8.3	5.1	6.1	6.8	8.8	9.4	6.8
Glu	9.8	14.5	14.4	16.3	11.7	8.0	11.7
Asp	12.4	14.8	9.1	9.8	8.4	10.0	7.3
Gly	4.7	5.3	2.9	1.9	3.8	5.4	4.5
Ala	1.0	4.0	5.3	6.2	6.1	9.0	6.3
Pro	4.9	5.3	5.0	5.0	8.0	4.8	6.4
Hyd			0.0	0.1			0

TABLE II

EGG PROTEINS

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Fish Eggs*	*Hens' Eggs Whole*	*Egg White*	*Egg Albumin*	*Egg Yolk*
Arg	7.0	6.7	5.9	6.0	7.4
His	2.4	2.4	2.5	2.2	2.5
Lys	8.0	6.9	6.4	6.5	7.1
Tyr	4.8	4.1	4.3	3.9	4.7
Try	1.0	1.6	1.8	1.5	1.6
Phe	4.8	5.8	6.0	7.1	4.9
Cys	1.5	2.3	2.6	1.8	1.8
Met	2.9	3.3	4.0	4.9	2.4
Ser		7.8	7.3	7.4	6.9
Thr	5.7	5.0	4.7	4.2	5.7
Leu	9.8	9.4	9.0	9.0	9.3
Iso	7.2	6.9	6.4	6.8	6.2
Val	7.3	7.4	7.8	8.1	6.8
Glu		12.6	12.8	14.9	12.0
Asp		8.2	7.6	9.6	5.6
Gly		3.6	3.7	3.5	3.5
Ala				6.9	
Pro		4.5	2.9	3.8	
Hyd				0.0	

TABLE III

HORMONES

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Insulin*	*ACTH*	*Thyroglobulin (Normal)*
Arg	2.9	8.3	9.4
His	5.1	1.3	3.6?
Lys	2.6	5.4	4.4
Tyr	11.9	3.1	3.4
Try	0.0	0.0	2.2
Phe	8.7	4.4	6.7
Cys	12.7	7.4	3.1
Met	0.0	1.7	1.3
Ser	5.5	5.2	10.8
Thr	2.5	2.9	
Leu	13.9	7.6	12.8
Iso	2.3	3.6	
Val	9.2	4.2	1.5
Glu	18.8	13.4	
Asp	6.9	10.8	
Gly	5.8	7.5	3.7
Ala	4.6	7.2	7.4
Pro	2.2	7.5	
Hyd	0.0	0.0	

TABLE IV

MILK PROTEINS

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Colostrum*	*Whole Milk (Various)*	*Whole Milk (Cow)*	*Casein (Cow)*	*Lact-Albumin (Cow)*	*β-Lacto-globulin*
Arg	4.5	4.0	3.5	4.2	3.1	2.9
His	2.4	2.6	2.7	3.0	1.8	1.7
Lys	7.2	7.4	8.0	8.2	9.7	11.9
Tyr		4.9	4.9	6.3	3.2	4.0
Try	1.6	1.5	1.3	1.5	1.8	2.3
Phe	4.1	4.7	5.1	5.8	4.0	3.8
Cys		1.5	0.9	0.4	2.7	3.0
Met	1.7	2.1	2.4	3.3	1.9	3.3
Ser		4.9	5.2	6.3	4.8	4.3
Thr	5.8	4.6	4.7	4.5	5.2	5.2
Leu	8.5	9.7	9.9	10.1	12.0	15.0
Iso	4.8	6.4	6.5	6.6	6.7	7.4
Val	7.4	6.5	6.7	7.4	5.3	5.8
Glu		19.4	21.7	23.6	17.7	19.8
Asp		7.7	7.5	6.5	11.1	11.7
Gly		2.1	2.1	2.1	2.5	1.7
Ala		3.6	3.6	3.1	7.0	6.8
Pro		8.9	9.2	12.3	4.7	5.2
Hyd		0.0	0.0	0.0	0.0	0.0

TABLE V

Muscle Proteins
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Crustacea*	*Fish*	*Fowl*	*Mammal*
Arg	7.6	6.6	6.7	6.6
His	2.0	2.9	2.0	2.8
Lys	11.1	10.1	7.7	8.5
Tyr	4.0	2.4	2.7	3.1
Try	1.0	0.9	1.0	1.1
Phe	4.0	3.8	4.1	4.5
Cys	1.9	1.2	1.0	1.4
Met	2.7	2.8	2.4	2.5
Ser		3.5		5.1
Thr	4.3	4.5	4.0	4.6
Leu	9.6	7.9	8.2	8.0
Iso	4.6	5.2	4.2	4.7
Val	4.3	5.5	4.1	5.5
Glu	15.5	12.0	16-18	14.6
Asp	9.3	8.6	10-11	8.0
Gly	6.8	5.4	5.7	5.0
Ala	5.8	6.1		6.5
Pro		5.1		5.0
Hyd				4.7

TABLE VI

Structure Proteins
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Collagens Gelatins*	*Elastins*	*Eukeratins*	*Silk Fibroin*
Arg	7.7	1.2	8.1	2.2
His	0.8	0.1	1.0	0.5
Lys	4.0	0.5	2.9	1.1
Tyr	0.9	1.9	4.5	9.6
Try	0.0	0.1	1.2	1.1
Phe	2.2	4.3	4.0	1.0
Cys	0.2	0.2	9.2	0.6
Met	0.9	0.2	0.9	0.1
Ser	4.0	0.9	9.3	10.1
Thr	2.6	1.1	5.6	1.3
Leu	3.2	7.6	7.6	0.9
Iso	1.6	3.4	3.9	0.9
Val	2.8	15.6	5.8	3.6
Glu	10.1	2.8	12.6	1.8
Asp	5.7	0.7	7.1	2.9
Gly	21.9	24.2	7.1	30.0
Ala	9.1	21.3	4.2	34.8
Pro	12.8	13.0	6.8	0.4
Hyd	12.4	1.5	0-9	

TABLE VII

TISSUE AND ORGAN PROTEINS
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Brain*	*Lens (Eye)*	*Heart*	*Kidney*	*Liver*	*Lung*	*Pancreas*	*Spleen*
Arg	5.7	9.1	5.6	5.7	5.7	5.1	5.3	5.4
His	2.4	3.6	2.5	2.7	2.6	2.5	2.6	2.6
Lys	6.3	4.7	8.1	7.4	7.5	6.9	7.2	7.1
Tyr	4.7	6.2	3.2	2.6	3.6	3.0	2.7	2.2
Try	1.3	2.8	1.4	1.3	1.6	1.8	1.9	1.2
Phe	5.1	8.4	5.0	5.1	5.1	5.1	5.3	4.5
Cys	1.7	1.6	2.2	2.1	1.7	3.3	3.2	2.7
Met	2.4	2.8	2.3	2.1	2.3	1.7	1.9	1.7
Ser	7.0	7.7	6.2	6.9	5.4	6.5	7.3	6.1
Thr	4.9	4.0	4.9	4.8	4.7	4.7	5.0	4.2
Leu	9.0	7.7	9.0	8.4	9.1	8.3	8.2	8.3
Iso	4.8	5.9	4.8	4.0	5.0	4.0	4.7	4.3
Val	5.4	5.3	6.0	6.5	6.1	5.9	6.0	5.6
Glu	11.7	14.7	13.9	13.1	11.8	11.4	11.5	12.2
Asp	10.0	9.2	8.4	9.1	8.0	9.7	7.3	7.5
Gly	6.4	5.1	5.2	5.1	4.6	4.1	4.6	4.2
Ala	6.4	5.0	7.8	5.9	6.7	6.2	7.0	5.0 ?
Pro	5.2 ?		6.1	6.5	5.0	5.8	7.1	5.5
Hyd			?	2.1 ?		3.7 ?	?	

TABLE VIII

WHOLE ANIMALS AND AVERAGE U. S. DIET
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

	Whole Animals			*Average U. S. Diet*	
Protein	*Fowl*	*Mammals*	*Invertebrates*	*From Analyses of Diets*	*Calculated from U. S. Diets (Block & Bolling, 1951)*
Arg	6.2	6.4	6.3	5.1	5.0
His	1.9	2.2	1.6	2.7	2.1
Lys	6.9	7.1	7.2	6.3	5.1
Tyr	3.2	2.8	3.0		4.1
Try	1.0	0.8	0.8	0.9	1.1
Phe	4.1	3.7	5.3	4.0	4.8
Cys	2.1	1.4	1.4		3.7
Met	1.9	2.1	2.2	2.1	
Ser			4.6		
Thr	4.1	3.9	4.9	3.5	3.5
Leu	7.1	7.0	7.3	7.1	7.9
Iso	4.2	3.7	4.3	4.8	5.2
Val	5.3	5.2	4.3	5.2	5.2
Glu	11.4	12.6	12.3		
Asp	8.9	9.0	11.4		
Gly	9.2	9.6	4.5		
Ala			6.6		
Pro			5.0		
Hyd	0.6				

TABLE IX

ENZYMES

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Lysozyme*	*Chymo-Trypsinogen*	*Pepsin*	*Papain*
Arg	12.0	2.8	1.4	7.1
His	1.1	1.5	1.1	0.8
Lys	5.4	7.7	1.5	4.8
Tyr	3.4	2.8	9.5	12.4
Try	7.8	5.6	2.3	4.2
Phe	2.8	3.8	7.0	2.6
Cys	6.9	4.6	2.2	2.6
Met	2.0	1.2	1.8	0 ?
Ser	6.9	11.3	12.1	4.7
Thr	5.3	10.7	10.0	3.3
Leu	6.3	9.3	10.1	5.3
Iso	5.3	5.8	9.7	5.2
Val	5.0	10.0	7.4	6.9
Glu	3.7	10.0	19.8	10.7
Asp	13.8	11.1	13.0	9.4
Gly	6.7	5.0	7.1	6.9
Ala	6.6	6.9	4.4	4.3
Pro	1.5	4.5	5.2	4.2
Hyd	0.0	0	0.0	0

TABLE X

VEGETABLES, NUTS, SEEDS

Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Potato*	*Cottonseed*	*Peas and Beans*	*Linseed Meal*	*Peanut*	*Soy-Bean Meal*	*Sun-Flower Meal*
Arg	5.3	12.1	7.1	9.4	10.9	7.0	8.6
His	1.8	2.7	2.7	2.2	2.5	2.5	1.8
Lys	5.3	4.4	6.1	3.5	3.5	6.6	3.3
Tyr	2.5	2.2	4.6	3.1	3.7	3.2	2.4
Try	1.1	1.3	0.9	1.4	1.2	1.2	1.1
Phe	5.1	5.4	5.2	4.6	5.1	4.8	4.5
Cys	1.2	1.6	1.2	1.5	1.4	1.2	1.6
Met	2.1	1.4	1.1	1.6	0.8	1.1	1.9
Ser	2.6	3.9	5.4	5.6	6.6	5.6	
Thr	3.7	3.4	4.0	4.1	2.7	3.9	3.4
Leu	6.2	5.7	8.1	6.4	6.1	7.6	6.4
Iso	5.1	3.6	5.3	5.0	4.0	5.8	4.8
Val	5.9	4.6	5.3	5.1	4.9	5.2	5.1
Glu	7.4	17.1	18.5	22.1	19.8	18.5	18.0
Asp	9.8	10.0	8.1	10.3	13.5	8.3	
Gly	1.9	3.9	4.6	5.4	5.0	3.8	5.0
Ala	6.1	4.0	4.1	4.6	5.3	4.5	
Pro	3.0	3.6	3.8	4.1	5.1	5.4	
Hyd							

TABLE XI

CEREALS, GRASSES, ETC.
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Alfalfa Meal*	*Barley Meal*	*Clover Meal*	*Corn Meal*	*Millet*	*Oat Meal*	*Rice Meal*	*Sorghum*	*Wheat Whole*	*Wheat Gluten*
Arg	4.9	4.9	3.0	4.4	3.3	6.9	7.9	3.4	4.3	3.6
His	1.8	1.9	1.9	2.4	2.4	2.2	2.5	2.5	1.8	1.9
Lys	5.0	3.6	4.0	2.7	2.4	4.4	3.4	2.0	2.5	1.6
Tyr	2.9	3.4	2.6	4.6		3.9	5.8	4.2	3.6	3.1
Try	1.3	1.2	1.8	0.7	1.7	1.2	1.2	1.1	1.2	0.9
Phe	4.2	4.9	4.4	4.5	4.0	4.8	4.7	4.8	4.4	5.1
Cys	1.6	2.4	1.6	1.6	1.3	1.2	1.1	1.6	3.3	2.0
Met	1.0	1.5	0.9	1.8	2.3	1.5	1.8	1.6	1.2	1.6
Ser	4.3	4.3	6.7	4.4		3.7			3.8	4.3
Thr	5.1	3.5	5.9	4.1	3.8	3.7	3.6	3.4	3.9	2.5
Leu	7.2	6.7	8.5	12.7	12.4	7.2	8.4	16.1	6.9	6.8
Iso	4.6	4.4	4.9	4.0	5.3	4.9	4.7	5.2	4.4	4.2
Val	4.7	4.7	7.2	5.3	6.3	5.3	6.3	5.6	4.5	4.3
Glu	8.0	19.5	11.6	18.4		14.3			31.4	30.7
Asp	14.9	4.9	9.4	12.3		4.1			3.8	4.8
Gly	4.8	3.6	5.1	3.5		3.6			3.4	4.0
Ala	5.5	4.3	6.0	10.0		5.2			3.0	2.1
Pro	4.0	8.1	3.1	7.2		4.9			10.3	11.1
Hyd										

TABLE XII

MICROSCOPIC PLANTS
Approximate Amino Acid Composition
Calculated to 16.0 gm. of Nitrogen

Protein	*Algae*	*Bacteria*	*Fungi*	*Yeasts*
Arg	7.3	6.0	5.3	4.4
His	1.9	1.8	4.6	2.3
Lys	6.7	5.3	4.4	6.7
Tyr	3.2	2.1	4.1	3.5
Try	2.0	0.8	1.5	1.4
Phe	5.2	3.8	4.0	4.0
Cys	0.5	1.9	2.6	1.1
Met	1.4	1.5	1.4	1.4
Ser	6.5	2.6	4.1	5.6
Thr	3.8	4.2	4.2	5.3
Leu	7.7	6.0	8.8	6.5
Iso	4.6	4.5	2.7	5.3
Val	6.2	5.1	6.3	6.0
Glu	9.7	14.3	45.3	11.7
Asp	8.5	11.6	18.3	8.3
Gly	5.0	3.5	4.5	4.1
Ala	6.8	6.0	6.9	8.5
Pro	5.5	2.3	5.5	4.2
Hyd				0.0

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